Synthetic Biology Guidebook

For iGEM High School

iGEM

Alberta Innovates Technology Futures

geekStarter

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1. INTRODUCTION

Emily Hicks

1. The iGEM Competition

Can a team of undergraduate students use today's scientific and engineering tools to build an organism that can solve a problem facing our world? This was the initial question posed by the International Genetically Engineered Machine (**iGEM**) competition. The goal was simple, to challenge students to become better tinkerers and problem solvers using the power of **synthetic biology**. That is, combining the power of engineering which builds solutions, with the science which seeks to understand how the solutions work. This approach is radically different from traditional research where undergraduate students rarely get to drive the designing, building, and testing of a research project of their own.

The iGEM competition originated at the Massachusetts Institute of Technology (MIT) in Cambridge, MA in 2003. It started as a one month long course where students were challenged to make a cell blink. The next steps involved expanding from MIT to other schools in the US in 2004. In 2005, iGEM saw its expansion on to the world stage with the addition of teams from Canada and Europe. In the next year, teams from Latin America, Asia and Africa joined. Since its inception, iGEM has been quickly evolving from a class on MIT's campus in 2003 to a competition with 234 teams from around the world in three different divisions in 2013. As iGEM expanded, there has been an ongoing push to move beyond just college students, expanding to include high school students and even community labs.

In 2011, iGEM offered for the first time an entirely separate division for high school teams to compete in. This aimed to provide high-school students a level playing field and the opportunity to interact with other students in their age group. For the high school competition the timeline runs as follows. Team project planning and registration November to January followed by project work from January to May, and then finalization of the project and the world high-school jamboree in June.

For the collegiate and entrepreneurial divisions the competition follows the college semester schedule. Project design and recruitment occurs from January to April, main project work takes place from May to August, while in September and October the project gets finalized in preparation for the competition. Typically there are two levels to the collegiate competition, the regional jamborees in October and the world jamboree in November.

As in the collegiate competition, high school teams need to prepare three major presentations. First, they need to develop a **wiki** - the website that fully describes their entire project from start to finish. Second, there is a 20-minute presentation plus a 5-minute question session in front of a panel of judges and other teams at the world jamboree. This presentation must run as a captivating story showing the world the work they have done. Finally, teams must present a scientific poster to the judges and other iGEM teams, which explains in greater detail the goals of their project and how each of the project components fits into the broader story.

2. Types of iGEM projects

Over the years, projects for the competition have been as diverse as the universities entered. At the collegiate level, teams are sorted into tracks depending on the type of project they choose to undertake. Although these tracks change slightly from year to year, some such as 'foundational advance', 'food and energy', 'environment' and 'health and medicine' stay the same. Projects are very diverse, from designing bacterial blood cells 'Bactoblood' to designing bacterial fuel cells and everything in between. In 2013 a collegiate team from Imperial College made bacteria capable of degrading plastic while a team from Taipei created a system to detect and protect honey bees from a dangerous pathogen. On the high school level, projects have been equally diverse. The winning team in 2013 from the University of Lethbridge developed a system to treat type I diabetes. This involved designing a genetic construct that could detect glucose levels and then produce and secrete insulin in response to low glucose levels. To do this, they needed to find a glucose sensitive **promoter**, put it into *E. coli* and then design a method for the insulin to be secreted out of the cells. In 2013, the third place team from Taipei developed a system to extend telomeres in order to prolong life. The project involved identifying genes and regulatory elements that could affect telomeres and then creating a design for how they could work together in a cell.

It seems the type of projects that teams can tackle is limited only by the imagination. In fact, because there are so few guidelines for it, picking a project can often be a major stumbling block for many iGEM teams.

3. Tips for Picking a Project

When deciding on a project, there are several things that need to be considered. The first is the general topic. There are two main categories of projects: foundational projects and application projects. While the aim of foundational projects is to add to the synthetic biology toolbox or change the way we clone, manipulate or even think about DNA, an application project makes use of synthetic biology to solve a problem. In 2010, the winning Slovenia team created **BioBricks** that allowed the use of DNA as a scaffold to dock **enzymes** and thus speed up reactions involving multiple enzymes. This is a great example of a foundational project. On the other hand, the Imperial College London team that placed 2nd in 2013 used bacteria in a bioreactor to break down different types of plastics: a great example of an application project. Both types of projects are allowed in the competition and can do very well. However, foundational advance projects can sometimes be more difficult to sell, especially if not as much data was obtained as was hoped, or if the parts didn't work as planned. In addition, sometimes teams make use of a great **human practices** component to compensate for setbacks experienced in other parts of the project. Finally, it can be more difficult to complete the human practices component for a foundational project as the implications of the project may be harder to brainstorm and it may be more difficult to engage the general public in what you are doing. While it can be very easy to get people excited about bacteria that clean up oil spills, explaining to the community a new and more effective method of cloning, while potentially important and innovative, may prove challenging. The students themselves may also have a more difficult time getting motivated about a foundational project. Although foundational projects can be very successful, it is good to keep in mind possible drawback as mentioned above. In the end, whatever you choose, think about how you would tell a story about it and share your work with others, as this will ultimately be what you will need for a good project.

A good place to start looking for project ideas is in the media. Have there been any hot topics this year that are relevant to your community? Are there certain pollutants that your community cares about or a disease that has been prevalent? Sometimes making use of current events in your area or beyond can help you tell a really great story. Another great place to look is in the scientific literature. Although access to publications can be challenging at times, this is something the mentors can help you with. Looking through the literature can help you figure out what scientists have already done about a certain problem. Sometimes, taking a recently discovered gene or promoter and making it into a BioBrick can result in a great project that is both feasible and novel to iGEM.

It is also important to consider the kind of expertise and resources you have access to. Picking a project about taking bacteria to Mars may be very cool; however it may be tough to talk to any 'experts' about it. Think about who you know and what they could help you with. These don't have to be scientists. For example, if you choose a project trying to detect some kind of chemical that affects crops, it could be very beneficial to talk to a farmer about the problems this chemical causes. If you need some scientific advice, for example from someone who studies a particular disease, you can think about contacting a university professor, using resources such as VROC to talk to other scientists or simply talking to your mentors. Often times there are tons of people willing to give you information or opinions that can help your project along.

Once you have a problem or idea in mind, take a look at previous iGEM projects. This can be done by browsing the wikis (websites) from each year, which can be found in a link on the right hand side of the iGEM home page for that year. Checking what other teams have done will not only ensure that you are not repeating what another team has done, but will also give you some inspiration. At all levels of the competition, but especially at the high school level, building off of previous iGEM work is encouraged. For example, a team may document plans of using a certain part already in the Registry but may not actually get the time to test it out. Testing this part could therefore be part a great iGEM project. Similarly, a team may have used a DNA part in a certain way but you may be able to think of another novel way to use it. For example, maybe an acid-sensitive promoter has already been used as a sensor for acidity, while you decide to use the same promoter for a completely different purpose- for example, to produce a **protein** of interest only when a strong acid is around.

It is important to look not only at other projects, but at the parts registry, too. How exactly to navigate the registry is covered in a later chapter. By browsing previously submitted parts, you could get ideas for natural extensions of what has been done already. Often times a part may already be submitted, but it may need another part to function properly. Submitting this second part and showing that the two parts work well together could be a useful project. Similarly, you may come across parts that were not submitted or that have problems. Fixing and resubmitting such parts could become part of your own project. Browsing through parts carefully can also save you time. For example, if you plan to use a common part such as green fluorescent protein (GFP), it's good to know what other teams may have already submitted using GFP. You may want to put GFP under the control of an **inducible promoter**. Looking through the registry, you will likely be able to find GFP with different promoters and **ribosome binding sites** already in the registry. This means that you may be able to use one of the already existing constructs and save yourself the hassle of constructing a new GFP part. Although just making use of an already existing part does not count as submitting something new, if you use an already existing part to create something new you can speed up the process and increase your chance of obtaining results.

4. Necessary Skills for an iGEM team

Because of the complexity and interdisciplinary nature of the projects, iGEM teams often involve students with a variety of interests and skills. Besides a keen interest in biology, other skills and interests are needed, too. In order to be successful, a team will not only need to do lab work but also design and build their wiki. Therefore, some team members may wish to focus on building a functional and attractive website, as this can be a time-consuming task. Some teams may use computer modeling or other types of computer simulation or programming. For example, in the past teams have designed simple video games to explain their project, or animations to show what is happening inside the cells. Although this is not mandatory at the high school level, if students on the team are keen, they can definitely pursue such topics.

Other important skills needed on an iGEM team are graphic design, public speaking and community engagement. The human practices part of the project for example, which we will go into more detail later on, asks students to think critically about the wider societal implications of their work. Human practices activities may include talking to members of the community, giving presentations to the

public (**outreach**) and producing essays, videos, blogs, etc. that examine some of these implications. Because iGEM puts a strong emphasis on this, students with an interest in engaging others or examining ethical issues can make a strong contribution to the project. Finally, iGEM teams, being self-funded, often require strong fundraising efforts. This could involve contacting potential corporate sponsors and designing promotional materials. Therefore, students keen to do these activities are also necessary on the team. In short, iGEM teams require a wide variety of skill sets and interests that go far beyond what happens in the laboratory. In fact iGEM provides a unique opportunity for students with different backgrounds and strengths to work together and it should not be advertised solely to 'science-focused' students.

5. Benefits of Involvement in iGEM

With this basic overview of the competition, you might be wondering why participate in iGEM? Is it really worth all the work and what can I expect to get out of an experience like this? In truth there are many things you can expect to get out of an iGEM experience. For high school students, one of the most exciting things they may take from iGEM is the chance to really see science in action. Many of the concepts necessary to know for iGEM overlap with content taught in high school biology and other courses. What many past students have loved, is being able to see firsthand how that content could be applied to solve a real world problem. Instead of only learning it in the classroom, they are also getting to directly apply it to a project they help envision. For previous many students, this hands-on experience was critical in directing them to future studies in science.

In addition to a more engaging experience in science, students also get the chance to gain a variety of skills that will empower them in their future endeavors. These are skills such as team work, leadership and taking ownership of their own project. Although working on an iGEM team can be extremely challenging, it gives students the chance to take control of a project and be responsible for seeing it through. Every year students rise to the occasion and students that you would not expect voluntarily take on leadership roles. Other students who were perhaps not as engaged in the classroom, find iGEM as something they can get excited about and can rejuvenate their interest in science.

iGEM is not just beneficial for students however. From an educator's perspective, there are benefits as well. In addition to seeing students develop skills and apply knowledge from the classroom, iGEM allows educators to see their students become more engaged in their own learning. iGEM demands students to go beyond what they learn in the classroom and challenges them to explore related material. This can not only benefit them in terms of iGEM, but can help them in mastering what they are learning in the classroom. Finally, iGEM often challenge educators to learn new skills alongside the students. Depending on what area project is in or what background educators have, they may find themselves just as engaged in learning about new topics.

Participating in the iGEM competition is a demanding and challenging activity. Although it can be difficult to fit in among other commitments, both for students and educators, we strongly feel that it offers tremendous benefit to both groups.

2. MOLECULAR BIOLOGY

THE BASICS

Magdalena Pop

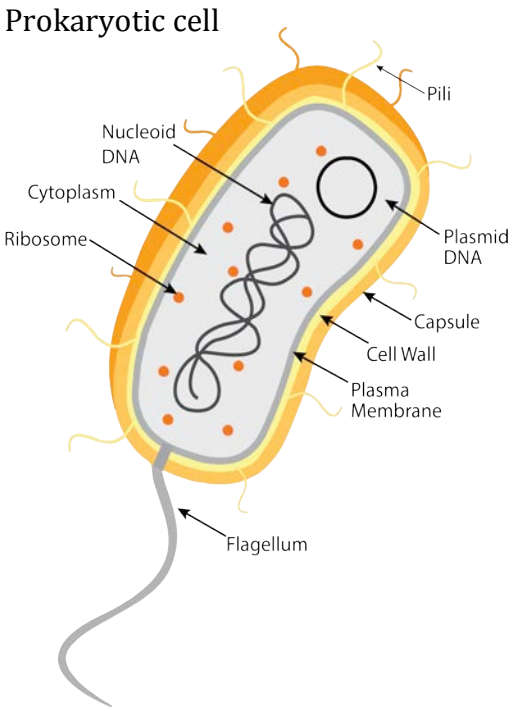
1. Cells

All live biological systems, whether natural or man-made (synthetic), are made of **cells**. A cell is the smallest unit of life, which means that a single cell is capable of all processes necessary to sustain life: metabolism, growth, reproduction, and adaptation.

Two components essential for any cell are its membrane and its DNA (Deoxyribonucleic Acid).

The **cell membrane** separates and protects the cell from its surroundings, while allowing for selected materials to enter and leave the cell. It is made of a double layer of **phospholipids** with **proteins** dispersed in it. This chemical composition results in just enough fluidity for substances to be able to pass through without loss of the integrity of the membrane. The outside of the cell membrane is studded with **carbohydrates**, which give specificity to cells.

Many small molecules pass through the cell membrane by **diffusion**, from the side of the membrane where concentration is higher to the side where it is lower, i.e. down their **concentration gradient**. Because it doesn't require energy this process is known as **passive transport**, and may or may not involve the assistance of proteins. Passive transport aided by membrane proteins is called facilitated diffusion.



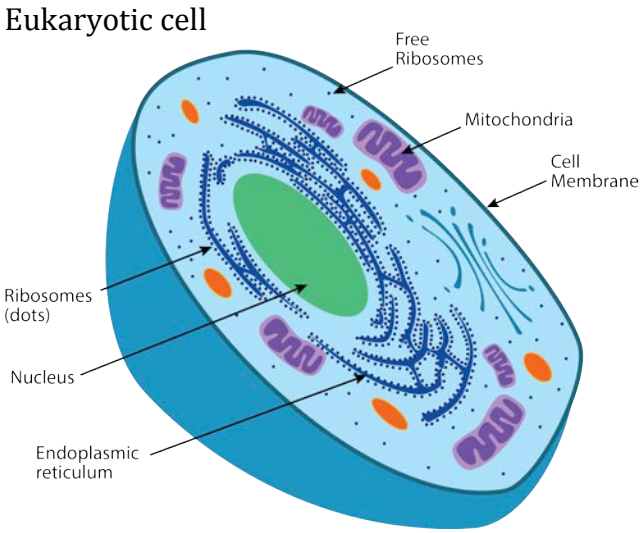
Sometimes small molecules must be moved against their concentration gradient. This is done by specialized proteins embedded in the cell membrane, whose job is similar to that of a pump pumping water uphill. Since it requires energy, this process is called **active transport**.

Big molecules (**macromolecules**) get in and out of the cell in transport sacs called vesicles.

The **DNA** contains all the information needed by the cell. More about the structure and function of DNA will be discussed in the next sections.

Cells with simple structures, which include little else beside a membrane and DNA, are called **prokaryotic cells**. They are the oldest type of cells on Earth. The most common prokaryotic cells are the bacteria.

Later in the evolution of life on Earth, a different type of cell emerged, which has a more complicated structure. Most notably, its DNA enjoys extra protection inside an organelle called a nucleus. Cells with a nucleus are called **eukaryotic cells**. Also unique to eukaryotic cells are additional organelles, such as the mitochondria and the endoplasmic reticulum. Each organelle carries out a specialized function for the cell. All organisms big enough to be seen with a naked eye, as well as some microorganisms, consist of eukaryotic cells.



2. DNA

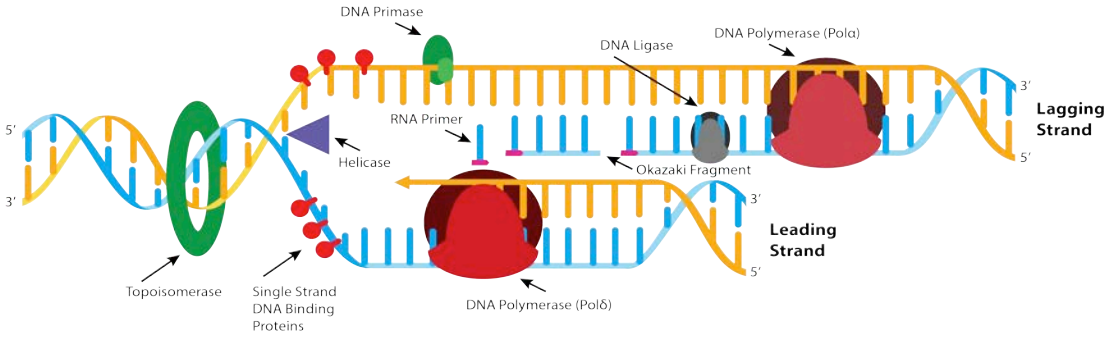
All cells store their information in DNA, which is packaged in one or several **chromosomes**. The information is coded as a particular sequence of chemical compounds, known as **base pairs**, along the length of the DNA molecule twisted into a **double helix**. Four chemicals form the basis of the DNA code: Adenine (A), Cytosine (C), Guanine (G), and Thymine (T). Due to their matching molecular structures, A pairs up with T, and C pairs up with G.

Semiconservative DNA replication

Each time a cell divides, the information stored in DNA must be passed along in its entirety to the two new daughter cells. For both daughter cells to carry the exact same DNA as the parent cell, the DNA molecule needs to be copied – or replicated – prior to cell division. Essentially two identical copies of the parental double helix must be produced. This outcome follows elegantly from the complementarity of A and T and that of G and C. Each of the **parent strands** is used as template and copied separately through insertion of **complementary bases** across from each base in their sequence. As a result, the two new DNA molecules are identical to the original DNA molecule and consist of both a parent and a **daughter strand**.

The replication of DNA results from the coordinated activity of several **enzymes**. It starts with the unzipping of the double helix at a specific sequence called **origin of replication**, which creates a replication bubble. The bubble then grows in both directions along the DNA molecule, as the main replication enzyme – the **DNA polymerase** – uses the exposed sequences of the parent strands as template to copy them into the daughter strands. Because of the antiparallel orientation of the two parent strands, the copying of one of them – the leading strand – occurs continuously, while the copying of its complementary strand – the lagging strand – has to be done in short fragments called Okazaki fragments. The Okazaki fragments are then joined into a continuous strand by an enzyme called **DNA ligase**.

DNA replication machinery



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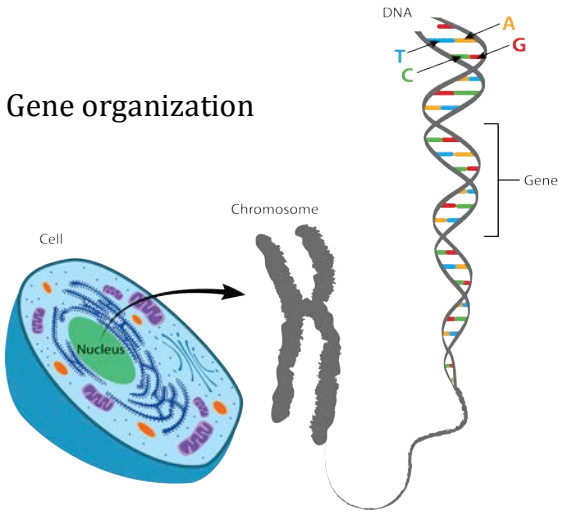
3. Genes

A particular DNA sequence containing a complete unit of information is called a gene.



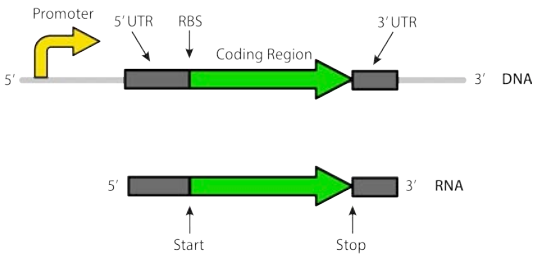
A gene includes the code for a functional product, which is typically a protein, together with sequences that serve regulatory functions, such as instructions for where to start decoding the information and where to end it.

A prokaryotic gene has a relatively simple organization. It starts with a **promoter**, which is followed by an uninterrupted **coding region** flanked by short stretches that play regulatory roles (**UTR**s).

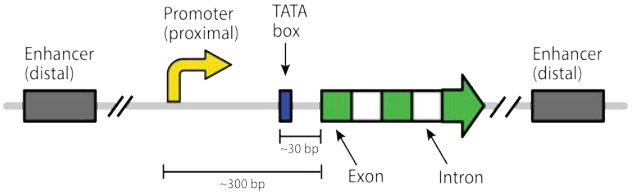
The organization of a eukaryotic gene is more complex. In addition to the promoter, a eukaryotic gene has extra regulatory sequences, such as enhancers, and its coding region is interrupted by non-coding sequences called introns.

A particular gene will serve its purpose when its DNA code is unlocked and used to make a protein. In this process, known as **gene expression**, the decoding of the information in the gene occurs in two main steps, transcription and translation. In transcription the DNA sequence is copied into a messenger RNA (**mRNA**). In translation the mRNA is translated into a product – a protein.

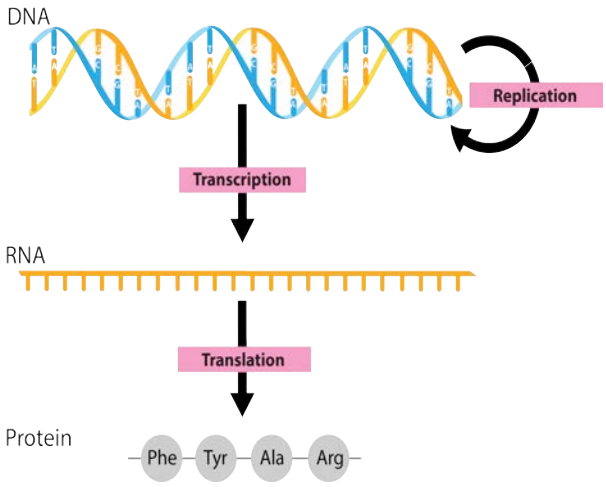
Typical bacterial gene



Typical human gene



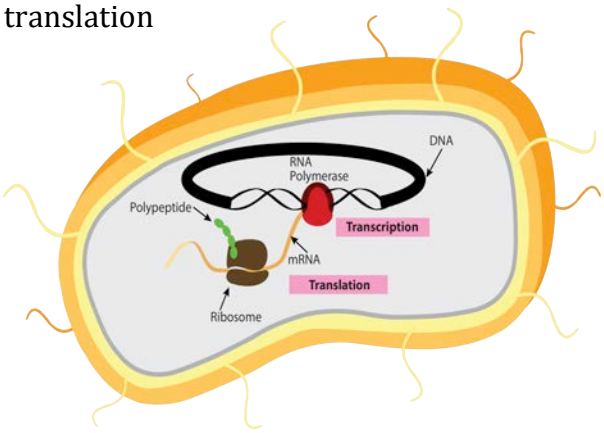
Gene expression



4. Transcription and Translation

Transcription is carried out by an enzyme called **RNA Polymerase**. After binding to the promoter the RNA Polymerase opens the double-stranded DNA and then copies its sequence of bases starting at a specified site. The copy is created according to the base pairing rules, e.g. a C is copied into a G, and a T is copied into an A. However, Adenines are not copied into Thymines. Instead, they are transcribed as Uracils (U), which are chemicals unique to **RNA** molecules.

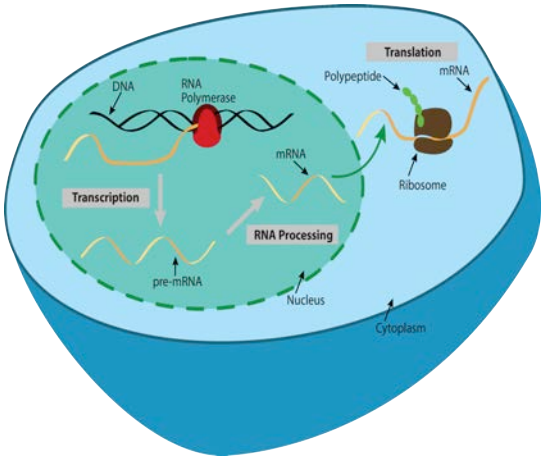
Prokaryotic transcription and translation



In a prokaryotic cell the resulting mRNA is immediately available for **translation.** This is because there is no nuclear enclosure and therefore the **ribosomes**, which do the translation, have easy and immediate access to the mRNA. Translation results in the production of multiple copies of the **polypeptide** (or protein molecule) encoded by the gene. To carry out the translation process ribosomes need **amino acids** – the main building units of proteins.

In a eukaryotic cell the process of transcription takes place inside the nucleus and it produces an initial, draft copy of the gene, called precursor mRNA (pre-mRNA). This draft copy is further edited (or processed) until the final mRNA is generated. It is the final mRNA that is sent outside the nucleus to be translated by the ribosomes.

Eukaryotic transcription and translation

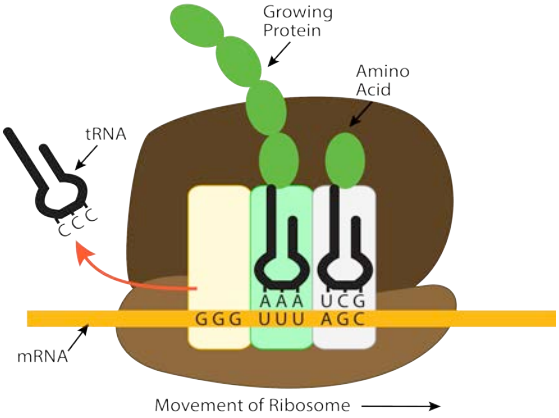


During translation the ribosomes read the mRNA sequence as a series of three-letter words, or codons. For each successive codon the protein chain is extended by one **amino acid**. The matching of codons to amino acids occurs based on the genetic code. Since there are more codons available (64) than there are amino acids (20), the same amino acid is represented by multiple codons. There is only one exception: AUG is the only codon for the amino acid Methionine, and it is found at the beginning of all mRNA molecules, i.e. it functions as the **START codon.** And three of the codons, UAA, UAG and UGA function as **STOP codons**, signaling the ribosomes to stop the translation.

The genetic code

Second Letter

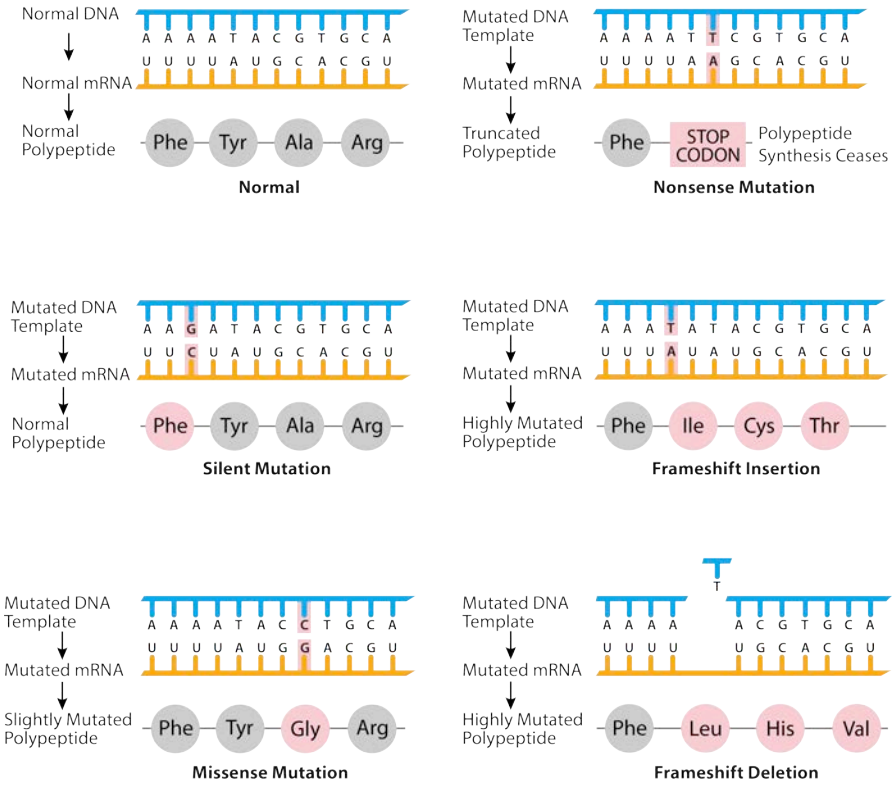
First Letter	U	C	A	G	Third Letter
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G



The amino acids cannot find their respective codons by themselves. Instead the matching is done by molecules of transfer RNA (**tRNA).** There are as many tRNA molecules as there are codons and each of them specializes in transferring one particular amino acid to the ribosomes. The feature of the tRNA molecule that enables the match is its **anti-codon** sequence, which is the exact complement to the codon for the particular amino acid that tRNA is carrying. For example, there are two tRNA molecules that transfer the amino acid Phenylalanine (Phe) to the ribosomes. The codons for Phe – UUU and UUC – are recognized by the complementary anti-codons – AAA and AAG, respectively – that these tRNA molecules have.

5. Mutations

Mutations are changes in the DNA sequence of a gene. Many different changes may occur, affecting one or multiple **base pairs** in the sequence. Some mutations change one base pair into another without changing the total length of the sequence (for example, changing an adenine to a cytosine). Other mutations eliminate or add one or more base pairs thus altering the length of the sequence. Ultimately what is important is the effect on gene expression, i.e. how the mutation affects the protein encoded by that gene.



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The effect that mutations have on gene expression can vary widely. Silent mutations are changes that have no effect whatsoever on the protein being made. Missense mutations result in a slightly altered protein, while nonsense mutations have more drastic effects ranging from a mis-functioning to a non-functioning protein.

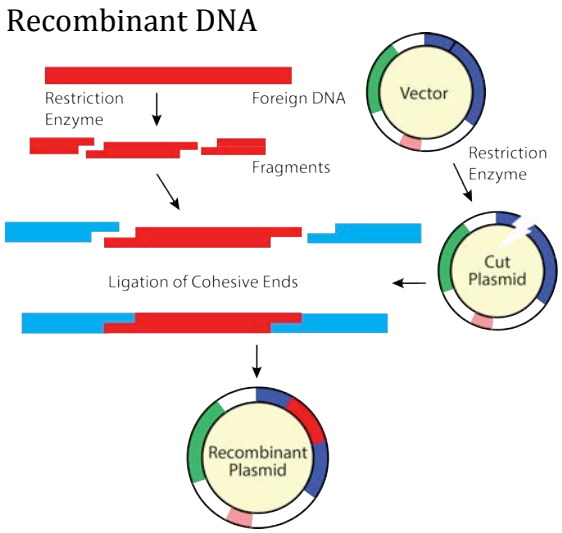
Sometimes mutations occur that shift the correct reading frame of a gene. Such frameshift mutations cause the ribosomes to misalign and to misread the mRNA. The mRNA is now read as a series of three-letter words different than the ones in the original code. Typically, a frameshift mutation has a drastic effect on gene expression, resulting in a defective, nonfunctional protein.

6. Bioengineering

This is a broad and rapidly evolving field of human endeavor, which takes an engineering approach to life sciences and aims to solve problems through redesigning biological systems and processes. Products of bioengineering are used in many different areas and industries, most notably in agriculture, medicine, energy and the environment.

The branch of bioengineering called **synthetic biology** produces life forms with useful properties by modifying the DNA of existing, natural organisms. Synthetic biology emerged about half a century ago when **recombinant DNA technology**, or molecular cloning, was first developed.

One of the earliest synthetic organisms created in the lab was an Escherichia coli (E. coli) bacterium, which carried a frog gene. The experiment involved the cutting of the frog gene DNA out of the frog chromosome followed by its insertion into the bacterial DNA.



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The procedure used in this early experiment is still central to synthetic biology, and it involves a few essential components: Scissor-like enzymes called **restriction endonucleases**, which cut DNA at specific sites, and a glue stick-like enzyme called DNA ligase, which glues back together the free ends of the cut DNA. Also essential to the success of the procedure is the use of a special kind of bacterial DNA, called **plasmid DNA**, which carries genes that render the bacteria resistant to particular antibiotics. This is important because it enables the experimenter to screen out the bacteria that do not carry the recombinant plasmid DNA, simply by adding the antibiotic to the growth medium. It also ensures that the bacteria will keep the plasmid in them, as long as antibiotic is around.

Resources

http://ghr.nlm.nih.gov/handbook/mutationsanddisorders/possiblemutations

3. SYNTHETIC BIOLOGY PRINCIPLES

Magdalena Pop

1. Introduction

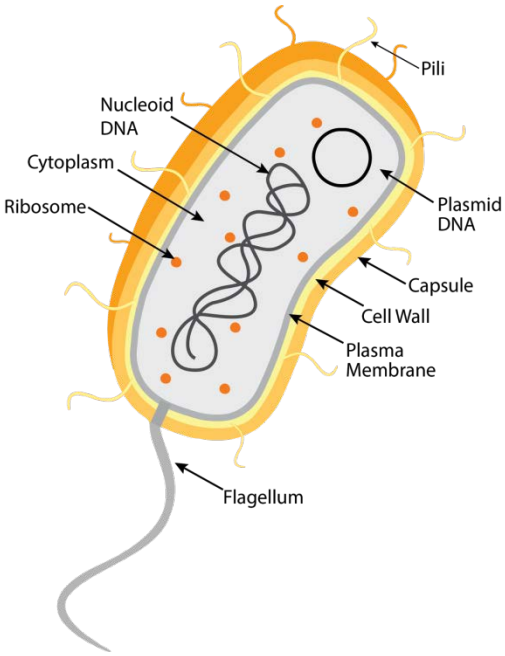
Specialists in **synthetic biology** regard **cells** as programmable machinery whose components work together in an intricate yet predictable manner. In their perspective, components of cells - such as **DNA** and **proteins** - interact and function similarly to the components of human-built machines. One can take apart all sorts of cells, study and catalogue their parts, and then design new ways to reassemble them to produce biological machines with novel functions. In the process, a growing collection and catalogue of DNA parts with specific functions – a Registry of **BioBricks** – emerged as an essential resource to reprogram living cells.

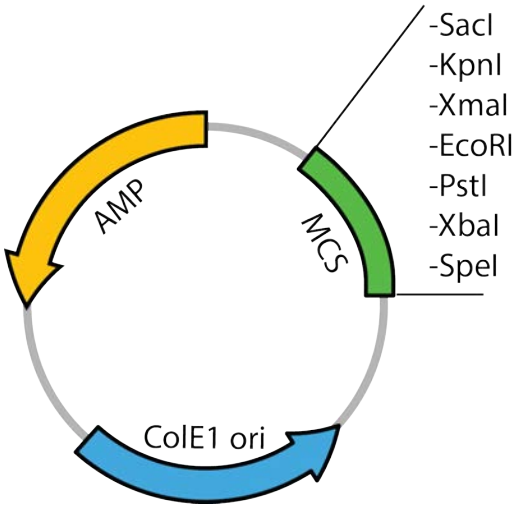
BioBricking gives many creative ideas a chance to become real. Such an idea, for example, was to design a biological source of light. The 2010 iGEM collegiate team from Cambridge (UK) brought this idea to life by putting firefly genes into E. coli, which resulted in E. glowli - engineered bacteria glowing in a variety of colours. The 2007 iGEM collegiate team from Berkeley (US) invented Bactoblood, which is made of bacteria redesigned to mimic the oxygen-carrying function of red blood cells. This product is meant as a safe and inexpensive alternative to real blood in transfusions To address environmental issues specific to Alberta's oil sands, the 2012 iGEM collegiate team from Calgary (Canada) created a bacterial system that does not only detect toxins, but can also convert them into usable products. This kind of biological tool offers many advantages in bioremediation. And the 2012 iGEM high school team from Heidelberg (Germany) envisioned iGEMs - wearable jewelry containing bacteria engineered to detect and measure UV radiation. Imagine how useful bracelets or rings would be if they didn't only serve as ornaments but also warned of excessive exposure to sun! These as well as many other examples of successful projects speak to the great innovation potential and promise of synthetic biology. But what are the main principles and methodologies of this emerging field? How does one plan a synthetic biology project?

2. How to Produce a New Protein in Bacteria

Say you wanted to make bacteria produce protein Z, a protein not normally made by bacteria. Protein Z could be a hormone such as insulin for example, or hemoglobin, the red oxygen carrier in human blood. How would you go about this project?

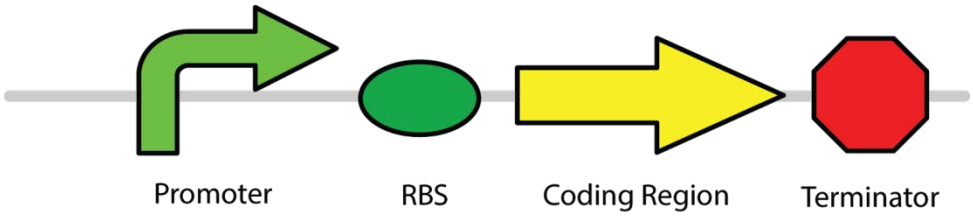
In order for bacteria to produce protein Z, the gene for protein Z – its DNA code – must be put inside the bacteria. It is useful to know that bacteria accept new DNA code relatively easily if carried by a plasmid. This is a small circle of DNA with its own **origin of replication**, which allows it to replicate independently of the **bacterial chromosome**. Plasmids also contain a gene for some **antibiotic resistance** (e.g. to Ampicillin), which allow for selection of appropriate cells containing the vector. Because antibiotics normally kill bacteria, only those that have the plasmid and are therefore resistant will survive in the presence of the antibiotic (see also section 5.10). The vector also contains multiple sites for **restriction enzymes** which help us cut the gene containing information for protein Z and insert it into the vector. The sites for restriction enzymes are locations in the **plasmid DNA** where engineering can be done, i.e. new DNA parts can be inserted. You will learn more about restriction enzymes later in this section.





In other words, your project will be to assemble the gene for the expression of protein Z in a plasmid, and then introduce that plasmid into bacteria. For this you will first want to find the DNA code for the **amino acid sequence** of protein Z - its entire **coding region** from the start to the **stop codon**. But remember that a gene is made up of more than just the coding region for a particular protein. Additional parts necessary for the gene to function are a **promoter** and a **terminator** – to mark the start and the end of **transcription**, respectively, and a **ribosome-binding site** (RBS) – needed for the correct initiation of **translation**.

Assembling your gene will be like assembling a circuit using the following DNA parts: (1) a promoter, (2) an RBS, (3) the coding region, and (4) a terminator. For this **genetic circuit** to function, i.e. produce protein Z, the order in which the four DNA pieces are assembled is of essence.



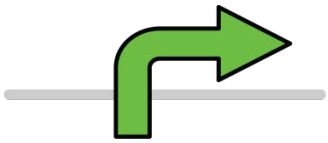
3. Promoters

How does the **RNA polymerase** locate a gene targeted for transcription? How does it know where to start copying the DNA to create the **mRNA** (the transcript)? This information is provided by the promoter sequence, which signals the RNA polymerase where to begin.

There are many different types of promoters. How are they different and how would you choose the promoter that suits your purpose the best?

If your aim is to make bacteria that produce protein Z constantly and regardless of the growth conditions, you will need to use one of the **constitutive promoters**. These are promoters that

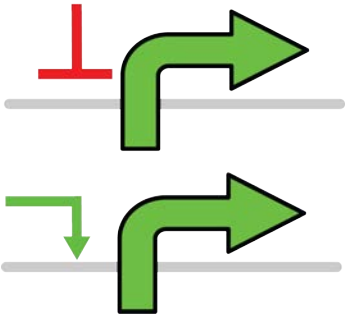
depend on little else beside the bacterial RNA polymerase. In other words, if the DNA is in bacteria, protein Z will be continuously expressed. You'll find examples of constitutive promoters in the Anderson promoter collection available on the registry of standard parts (see The Registry section). While all these promoters produce constitutive expression, each of them results in a different amount of protein. Keep in mind, while producing a large amount of your protein might give you better results, making your cells produce a large amount of protein all of the time might sometimes be bad for their health, resulting in slower growth.



Promoter

How about if you wanted to control the expression of protein Z in the bacteria? In that case, you would look for a promoter that can be switched on or off by a specific agent, e.g. a certain substance, or temperature. **Controllable promoters** exist that are either positively or negatively regulated. The activity of positively regulated promoters increases in the presence of certain **inducing agents**. The activity of negatively regulated promoters decreases in the presence of specific **repressors**.

Promoter Types



Some common examples of controllable promoters are the LacI promoter (pLacI), the araBAD promoter, or the **T7 promoter**. In the absence of an inducing agent, the activity of the LacI promoter is blocked (or repressed) through the binding of a repressor protein. Transcription of genes from the promoter will not occur. When an inducing agent is present – e.g. Lactose or **IPTG** (Isopropyl – beta-D-thiogalactoside) – the repressor becomes inactive and the promoter is switched on. For the araBAD promoter the most common inducer is arabinose. The T7 promoter needs **T7 RNA Polymerase** – a protein found in the T7 bacteriophage (a bacterial virus) – to get switched on, therefore you must make sure your cells express this protein as well for this promoter to work.

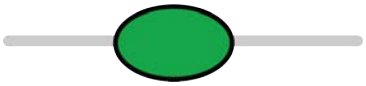
4. Other DNA Parts Essential for Gene Expression

In addition to a suitable promoter and the protein coding region, your circuit must also include an RBS, and a terminator.

As suggested by the name, ribosome-binding sites (RBS) are sequences where the **ribosomes** - the translation machinery - attach in preparation for making the protein. Without an RBS sequence, the

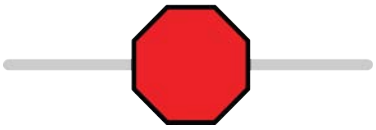
ribosomes would not be able to position themselves properly onto the **mRNA transcript** and, as a result, the translation of the mRNA into protein would fail. RBS sequences are typically short and they are found only a few **nucleotides** upstream of the **start codon**.

RBS BioBrick Symbol



Whereas an RBS is needed for proper translation, a terminator sequence is a part required in transcription. Once again, the name says it all! The particular sequence of nucleotides in a terminator causes the DNA at the end of a gene to fold, which blocks the progress of the RNA polymerase and effectively terminates transcription.

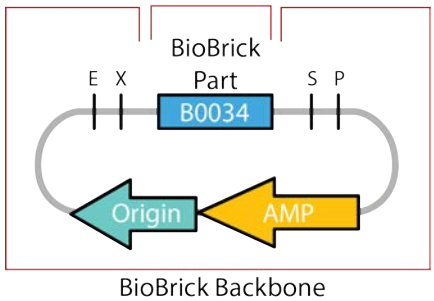
Terminator BioBrick Symbol



5. Designing the Genetic Circuit

Let's assume that all four DNA parts are available in the Registry. This usually means that all four (or more if you are producing more than one protein) are available as BioBricks carried by **plasmid vectors**; some may even be pre-assembled in the same plasmid. For example, we can assume that a construct made of an RBS – Protein Z coding region – Terminator has already been pre-assembled or BioBricked in a plasmid. We will call this plasmid A. And we will assume that the appropriate promoter is present as a BioBrick in plasmid B.

BioBrick Plasmid

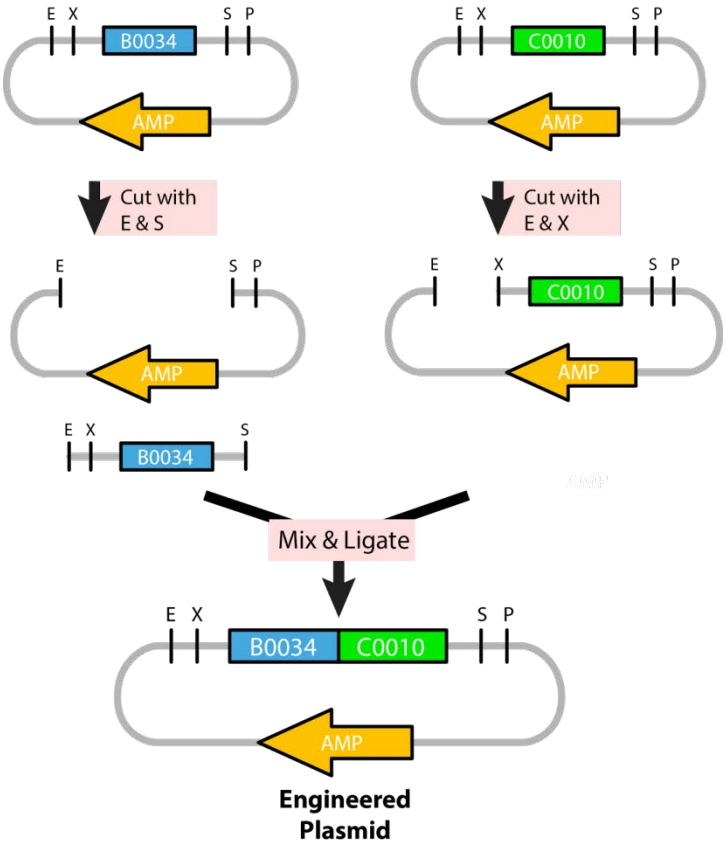


BioBrick Backbone

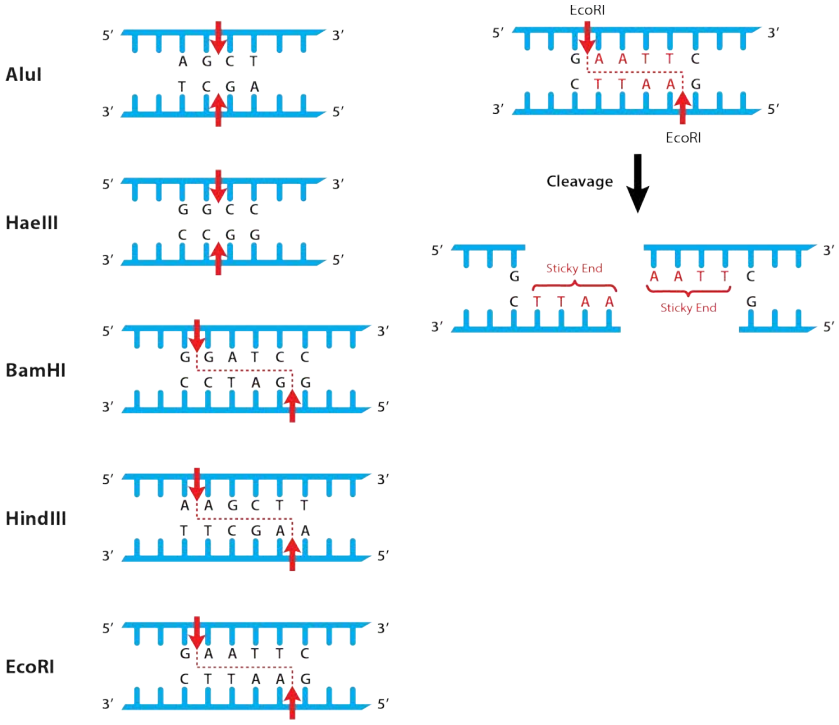
As long as both plasmid A and B are available, you are ready to build the complete genetic circuit for the expression of protein Z. You have two options. One option would be to cut out the promoter from plasmid B and insert it in front (or upstream) of the construct in plasmid A. Alternatively, you could

cut out the RBS – coding part – terminator construct from plasmid A and insert it after (or downstream from) the promoter in plasmid B. We'll go with the first option.

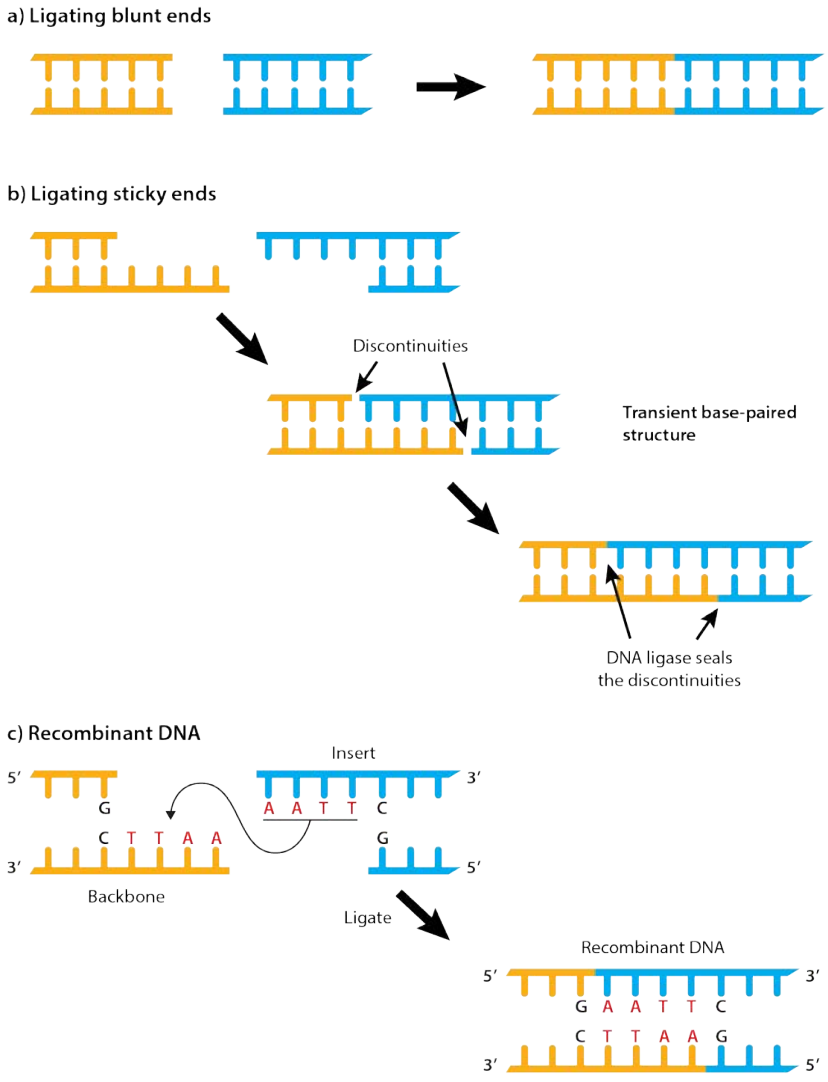
6. Assembling the DNA – Cutting and Ligating



To cut the plasmids you will need restriction enzymes, which are scissor-like protein molecules specialized in cutting DNA (see also section 5.8 and Appendix 1.8 for protocol). There are many different restriction enzymes, and what makes them useful is that each of them cuts a unique DNA sequence with palindromic properties, which means that the sequence reads the same in both directions. Restriction enzyme cuts are either **staggered** – producing sticky/cohesive ends – or straight – resulting in **blunt ends**.



Whether sticky or blunt, free DNA ends resulting from restriction can be joined or glued together later by an enzyme called **DNA ligase** (see also section 5.9 and Appendix 1.9 for protocol). Usually sticky ends are easier to join because the base-pair complementarity between the single-stranded overhangs favors the **ligation**.



Ends cut by the same restriction enzyme can always ligate together. Ends cut by different restriction enzymes cannot ligate unless they are blunt or their overhangs are complementary. For example, XbaI (X) and SpeI (S) are two different restriction enzymes whose cuts are complementary and will stick together in ligation. That is why these two restriction enzymes are used frequently for the assembling of BioBricks.

XbaI 5’ – T^ CTAGA – 3’

3’ – AGATC^T – 5’

SpeI 5’ – A^CTAGT – 3’

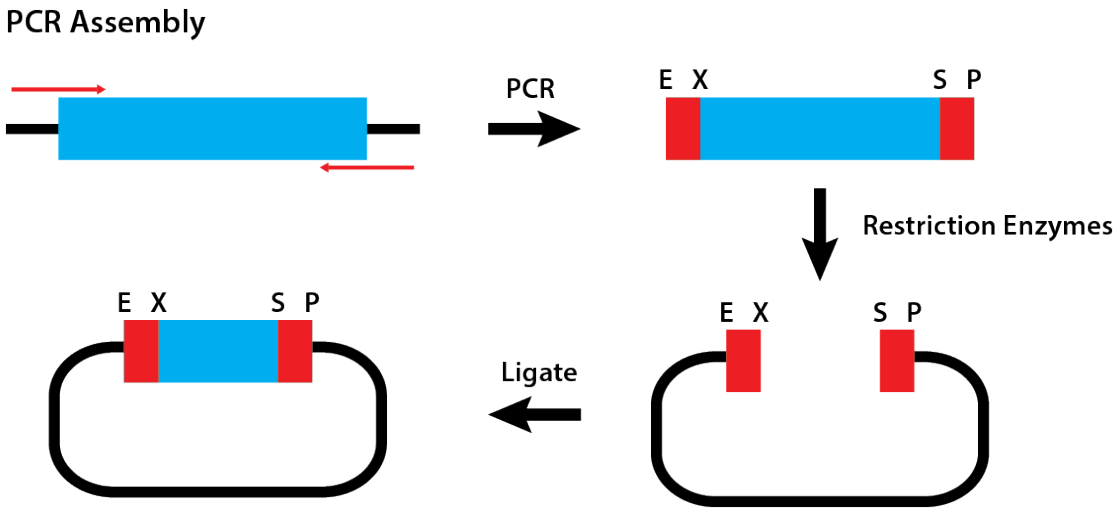
3’ – TGATC^A – 5’

Going back to your project now, the promoter will be cut out from plasmid B using restriction enzymes E (EcoRI) and S (SpeI), while plasmid A will be opened by cutting it with E (EcoRI) and X (XbaI). The E sticky ends of the two parts will then join together in ligation, and so will the S and X sticky ends (see above). This will result in the desired genetic circuit, with all four necessary parts for

the expression of protein Z assembled in the correct order within the plasmid A backbone. The newly engineered plasmid will then have to be added to the bacterial cells via **transformation**. You will learn how to do transformation of bacteria in the Methods section 5.10 and the Appendix 1.10 protocol. Finally, to ensure that the project has been successfully completed, you will need to verify that the transformed bacteria are indeed capable of expressing a functional form of protein Z.

7. Making a New BioBrick Using PCR

What if the gene for protein Z is not available as a BioBrick in the Registry? In that case you will need to make the BioBrick for protein Z yourself. For this you will use a method called Polymerase Chain Reaction (**PCR** – see Methods section). This method will enable you to select the gene for protein Z out of a DNA pool, and amplify it. How do you find your target gene? If you know its DNA sequence, it's easy. You place detective-like DNA molecules called **primers** in the DNA pool. These are short fragments of single-stranded DNA that match the start and the end of your target gene only and nothing else in the DNA pool. The primers will be able to find their matching complementary sequences, bind to them, and prime – or initiate – the copying of the in-between gene. The power of PCR is that – in a very short time – only one copy of the target DNA sequence can turn into millions of copies.



But what do you do with those many copies? Remember that your aim is to BioBrick the gene, which means that you want to insert it (or paste it) in a plasmid. This requires that your gene is flanked by suitable **restriction enzyme sites**, to allow it to stick into an opened plasmid. Meeting this requirement is simple: when you design or choose the primers for PCR, in addition to sequences matching your gene make sure to fit them with appropriate restriction enzyme sites. This will allow you to restrict the PCR product and conveniently ligate it in a destination plasmid.

8. BioBricking Using the 3A Assembly Method

Traditional assembling of DNA parts takes fairly long. Why? Because ligation requires that DNA parts first go through a time-consuming purification procedure called gel extraction (see Methods). This purification gets rid of any DNA pieces that you don't want to have in the final construct. If no purification is done after cutting, and all DNA pieces are left together, chances are high that ligation

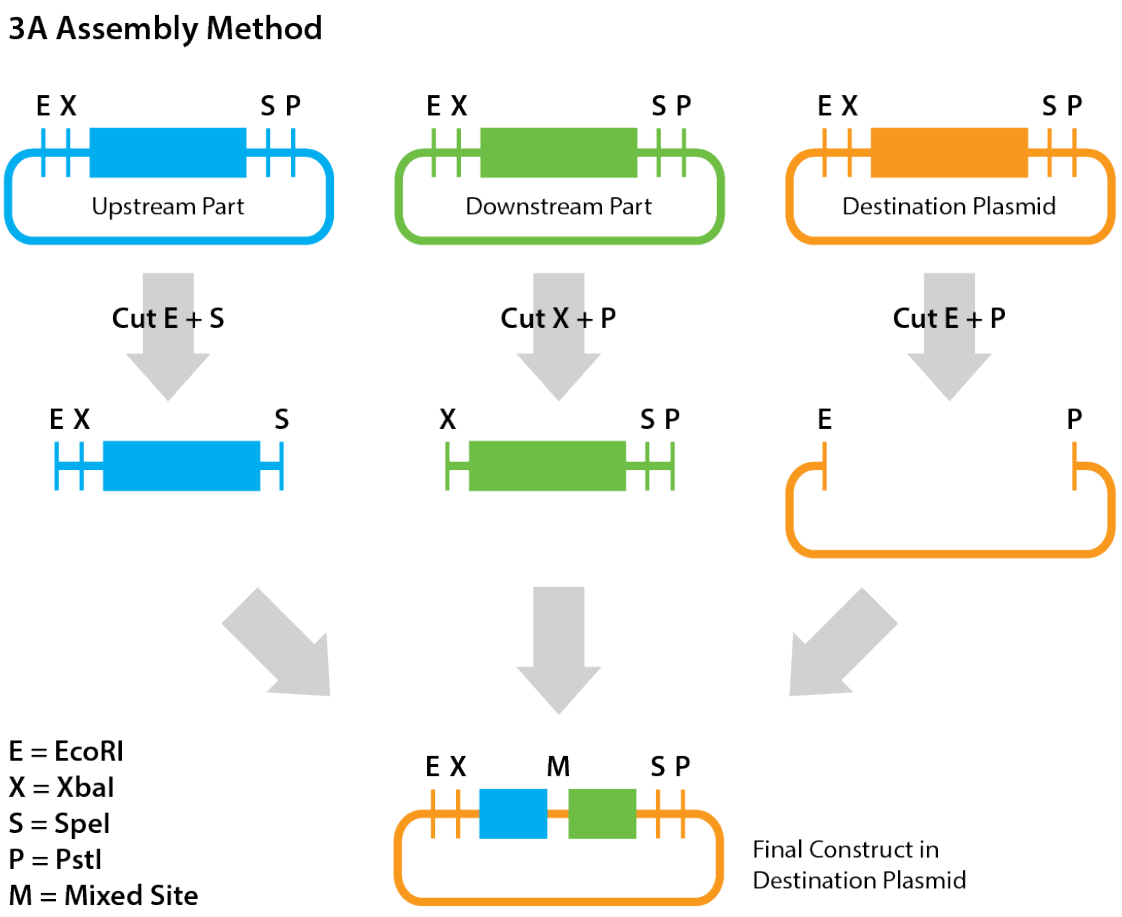
will regenerate the original plasmid along with the new construct. And because both the original and the newly constructed plasmid have the same antibiotic-resistance, you can't screen out the original simply by throwing a new antibiotic in the growth medium.

To eliminate the need for gel extraction and save time, an alternative method was developed called the 3A Assembly method.

In this method, the final construct is assembled in a destination plasmid whose antibiotic-resistance gene differs from those carried by the original plasmids. Three distinct antibiotic-resistance genes (3A is short for 3 Antibiotics) are thus involved, two found in the original plasmids (green and blue in the figure below) that provide the parts, and a third in the final, destination plasmid (orange). This means that right after restriction all the cut parts along with the empty plasmids can be thrown together in the ligation mixture, without prior separation by gel extraction. Although ligation will result in several plasmids, all of them except for the correctly assembled one will be screened out. How? By making sure to add the third antibiotic to the growth medium. This third antibiotic corresponds to the resistance of the destination plasmid, and will therefore only allow cells with this destination plasmid to grow.

The workflow of the 3A assembly method is shown in the figure below, and consists of the following steps:

1. Restriction digestion - See explanation in section 5.8 and protocol in Appendix 1.8
2. Ligation - See explanation in section 5.9 and protocol in Appendix 1.9
3. Transformation - See explanation in section 5.10, and protocol in Appendix 1.10



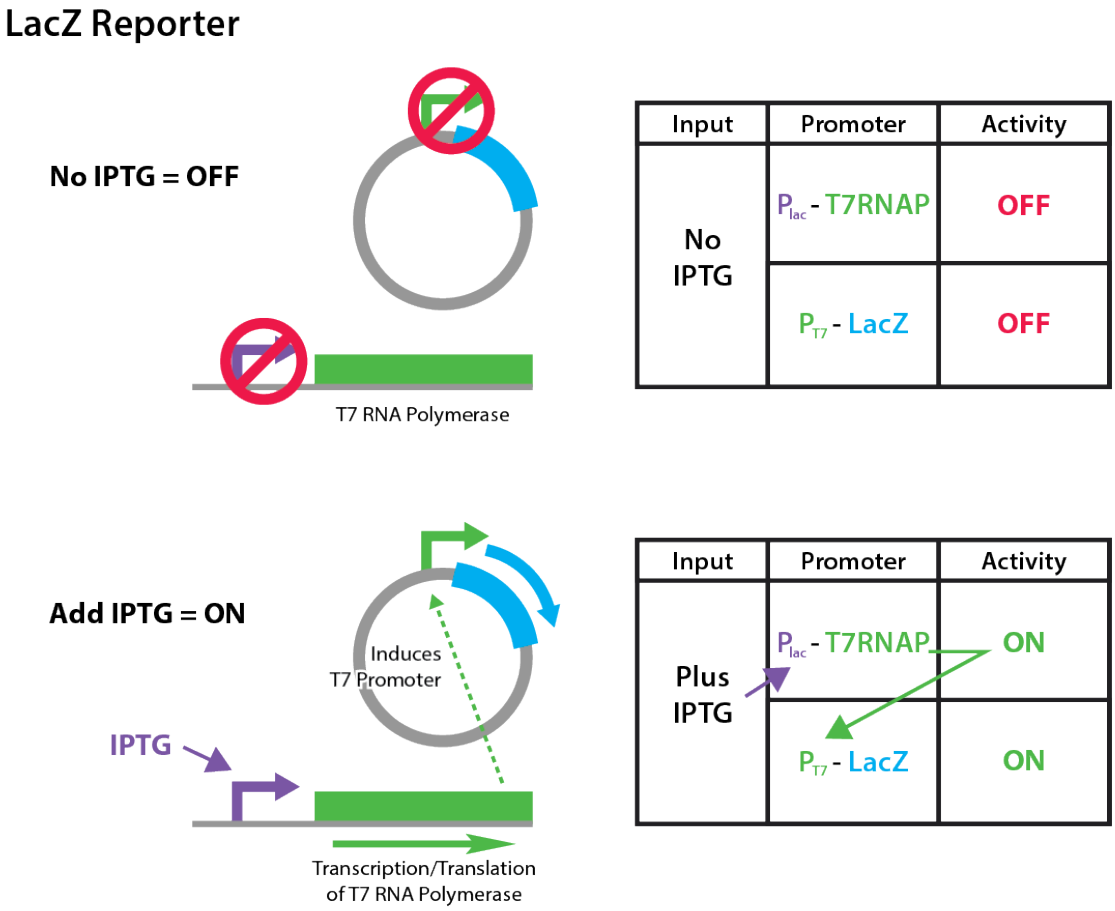
9. Reporter Genes

If you designed a new genetic circuit where a gene's expression is controlled by a novel regulatory system, a fast way to check that the new system works as expected is by testing it with a **reporter gene**. A reporter gene is a gene that produces an easily detectable output – e.g. a colored or fluorescent product – and can provide you with a quick report on how well your circuit functions.

Say, for example, that you wanted to place your gene of interest (e.g. the gene for protein Z) under the control of the T7 promoter. As described above, the T7 Promoter needs the T7 RNA Polymerase to become active. And where does the T7 RNA Polymerase come from? In your circuit design, it comes from the gene for T7 RNA Polymerase placed under the control of the LacI promoter. Your plan is to switch on the LacI promoter with IPTG, which will make T7 RNA Polymerase, which will activate the T7 Promoter, which will finally make the protein you want (Protein Z). In other words, your target output response (production of protein Z) is expected to occur every time the T7 promoter is switched on by input of T7 RNA Polymerase from the IPTG-induced LacI promoter.

To test that your system works, instead of the gene for protein Z, you would initially place a reporter gene downstream from the T7 promoter. You could for instance use the gene for beta-galactosidase as a reporter. And, in short, you will know if beta-galactosidase was expressed if the bacteria turned blue. With no IPTG there would be no T7 RNA Polymerase to switch on the reporter and the bacteria

should be white. Adding IPTG should switch on expression of T7 RNA Polymerase, which in turn will switch on expression of the reporter and turn the bacteria blue.



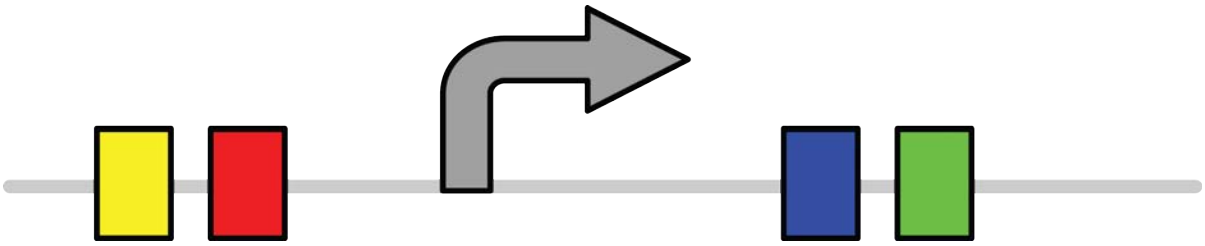
The bacterial gene for beta-galactosidase is one of the earliest reporters used in bioengineering. More recently, other genes useful as reporters were discovered, such as a jellyfish gene that makes a green fluorescent protein (GFP), a firefly gene for a bioluminescent protein, and various genes from corals coding for proteins of different colors. Where beta-galactosidase requires you to add a chemical to your cells (X-Gal) in order to see the blue color, these proteins will color your cells as soon as they are made by the cells.

4. THE BIOBRICK

Emily Hicks and Lisa Oberding

1. What's a BioBrick?

A very important component of the iGEM competition is the **DNA** pieces that are generated. These are known as **BioBricks**. BioBricks are DNA 'parts' that have some sort of function. First proposed by Tom Knight in 2003, there are three main components of a BioBrick. There is the 'part' sequence that encodes for the **protein** or regulatory device that we are interested in. This could be a sequence encoding the gene for green fluorescent protein, a sequence encoding a **promoter** element or a **terminator**, etc. This 'part' sequence is flanked by specific sequences of DNA on either end. These specific sequences are called cut sites, as they are recognizable by certain **restriction enzymes** whose function is to cut DNA. A BioBrick has three of these restriction enzyme sequences on each side of the part sequence. These sites include: *EcoRI*, *NotI*, *XbaI*, *SpeI*, and *PstI*. A schematic of a BioBrick can be seen below.



It's important to note that the three cut sites that come before the gene are called the **prefix**, while the three sites that come after are called the **suffix**. The 'part' sequence, together with this prefix and suffix sequence together make up what we call a BioBrick.

2. Why are BioBricks important?

BioBricks are important for two main reasons: standardization and modularity. By creating BioBricks, teams then test these BioBricks and find out certain parameters for them. For example, they may test a BioBrick promoter and find that it has high activity levels in the presence of high pH, and low activity levels in the presence of low pH. By having a 'standard' part that they are testing, other people can use this information to use that promoter in a different application. The hope would be that the part would behave exactly the same way as it is a 'standard part' (we know of course that science is rarely this straight-forward however!). The other reason is modularity. By having this standard for BioBricks, there is a simple method to put these parts together. This method is called the BioBrick assembly standard. In brief, if we wanted to put together two parts: part A and part B, there are two approaches we could take using this assembly standard. First we have to figure out which part needs to go upstream (in front) of the other part. If we're putting together a promoter and a gene for example, we would want the promoter to come first to enable transcription of our gene. With that in mind, we have two choices: we can use the promoter as our vector, and we can insert the

gene directly downstream of the promoter, or we can insert the promoter directly upstream of the gene, using the gene as a vector. The details of how this is done are covered in much more detail in the protocols section.

This assembly method allows us to string together single BioBrick parts and build increasingly complicated **genetic circuits**. Using a promoter BioBrick, a BioBrick encoding green fluorescent protein and a terminator BioBrick for example, it is possible to string these three basic parts together to build a simple genetic circuit, termed a 'composite' part. Teams can use basic parts contributed by other teams, couple with their own novel parts to create new genetic circuits.

3. Limitations to the BioBrick Assembly Method

Although the BioBrick assembly method makes it, in theory, easy to put BioBricks together, it does have several shortcomings. With its very nature, it can be difficult to deal with more 'abnormal' parts. For example, if you needed to create a fusion protein, where two proteins were put together, the BioBrick method needs to be altered. This is because the scar sequence that sits between the two parts and prevents them from coming together during any subsequent construction steps, also encodes an in-frame **stop codon**, essentially halting **translation** of the second protein in the fusion. Another example is if you were using a large gene and creating a new BioBrick, it would be necessary to remove all the 5 BioBrick cut sites, often called 'illegal' cut sites from this gene sequence. This means that in order to submit your new BioBricked gene, you would need to change any naturally occurring *EcoRI*, *NotI*, *XbaI*, *SpeI* or *PstI* sites in the gene. This is generally done by introducing a silent **mutation** to change the cut site without interfering with the **amino acid** code. Although this is completely doable and relatively easy if you were to synthesize your parts from a synthesis company such as IDT, it can be time-consuming if you are not using synthesis.

Although there are distinct advantages of the BioBrick assembly method, it is however, not always the most efficient way of putting DNA pieces together. In recent years we have seen the emergence of a variety of different assembly strategies such as Golden Gate or Gibson assembly. Many labs and even iGEM teams use these methods. Although we will not go over the details of these other methods, it is important to note that they do exist and should your team consider putting together a large number of parts, this may be something to consider. It is important to note however that you are still required to submit BioBricks. As such, even if you choose to use another assembly method, as many teams do, you must carefully design your parts so that the final product will be a BioBrick, irrespective of how you put it together. Another thing to note is that other methods, although sometimes more efficient, do lack the simplicity that the BioBrick method offers and often require additional reagents to be purchased, which can increase cost.

4. Other Standards

Although the BioBrick is the standard used in iGEM, and arguably one of the more widely-adopted standards, it is not the only standard around. Many labs and iGEM teams have proposed their own ideas for standardizing and assembling parts. In the registry, the BioBrick standard is often called RFC [10]. There are a few other standards that the registry also accepts however and you should be aware of these as you browse for parts. Many of these involve slight modifications to the BioBrick prefix and/or suffix. As such, digestion with different restriction enzymes (compared to the normal EcoRI, NotI, XbaI, SpeI, PstI) are used to assemble these parts. These include RFC[12], RFC[21], RFC[23] and RFC[25].

RFC[12], also known as BB-2 standard, is designed specifically for assembling multiple proteins. It allows you to assemble proteins without introducing either the traditional BioBrick scar site which is eight **base pairs** and as such introduces a frameshift, or the alternative scar sequence used for

proteins in RFC[10] which introduces a stop codon (scar sequences are discussed in detail further on). Although there are parts in the registry which are compatible with RFC[12], it is not widely-used. RFC[21], also known as the Berkeley Standard, is also used for protein assembly. This standard however used alternative restriction enzymes, BamHI and BglII. As BglII is not a heat-in-activated enzyme, the normal assembly method described later on cannot be used for this standard. RFC[23] is known as the Silver Standard and was designed specifically for fusion proteins. In this standard, the BioBrick prefix and suffix are each shortened to allow for a six base pair scar. This six base pair scar allows for the two proteins being joined to both remain in-frame. It should be noted however that the shorter, six base pair scar does introduce an arginine codon which does result in the protein degrading faster. Finally, RFC[25], also known as the Freiburg standard, includes modified prefixes and suffixes, again to allow for in-frame assembly of two proteins. When browsing through the registry, you can identify if a part is compatible with a certain RFC by looking at the colored boxes that appear on the main page of the part. These boxes have number sin them corresponding to certain RFCs (10, 12, 21, 23 and 25). If these boxes are green, this indicates that the part is compatible with that RFC, while red indicates that it is not. It should be noted however that 'being compatible' only means that this part does not have restriction sites in its sequence that correspond with the restriction sites in the prefix/suffix of that particular RFC ("illegal cut sites"). It does not however, indicate that you could use the standard BioBrick assembly method to put this part together with a BioBrick part.

In addition, to these registry-accepted standards, over the years many iGEM teams and other groups have suggested completely different standards that are not always compatible with the BioBrick standard. These include standard such as BglBricks (1) and BioBytes (2). Although some of these address quite well the limitations of the current BioBrick standard it should be noted that iGEM will not accept these. As such, your parts will still need to be in BioBrick form to count for the competition. It is important to watch for these other standards however as occasionally there are parts in the registry that follow these standards instead. For example, you may find a BglBrick part that you wish to use. As it does not possess the standard BioBrick prefix and suffix however, you cannot use the normal BioBrick assembly method to join it with another BioBrick. As such, you may need to use an alternative cloning strategy or replace the BglBricks prefix and suffix with the BioBricks ones via polymerase chain reaction.

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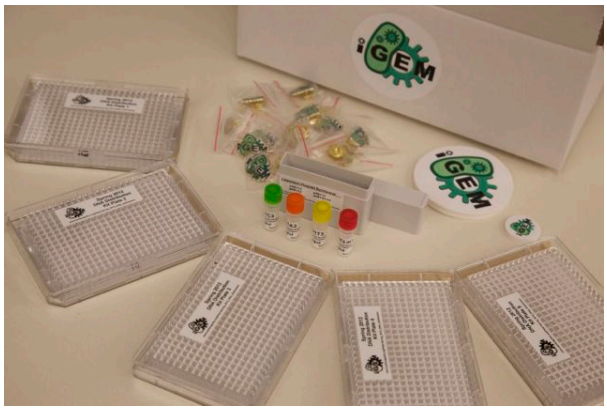
2. Armstrong, K., Bennett, E., Buchko, M., Cortes, O., Dao, A., Davidson, U., Fedor, J., Garside, E., Jahns, S., Leung, E., Lloyd, D., Nguyen, E., Paquette, M., Paul, A., Pon, J., Ponomarev, A., Robinson, K., Rodway, J., Wiltshire, Z., Yau, J., Maclagan, J., Ridgway, D. and Ellison, M. (2010). Igem 2009: The BioBytes Method. *Eureka*. 1(1): 5-6.

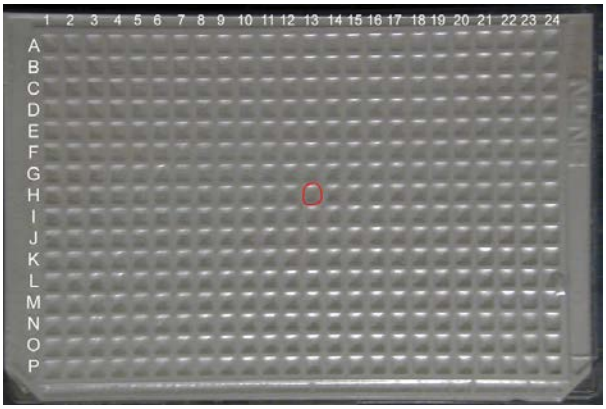
5. METHODS

Sutherland Dube and Zak Stinson

1. Using DNA distribution plates: Rehydration of registry DNA from kit plate
See Appendix 1.1 for protocol

Before we start doing any assemblies, we first need parts to put together! Every year, iGEM sends out a collection of the best-characterized and most useful parts that have been sent to them by teams in the past. These parts come in the form of the Kit Plates.





In order to use the parts that are sent in the Kit Plates, you need to know where to find them. The Kit Plates each contain 384 parts in wells organized in a coordinate system. Each well has a letter and a number associated with it (for example H13, as shown in the above picture).

To get a specific part from the Kit Plate, look up that part's coordinates on the iGEM DNA Part Libraries (http://parts.igem.org/cgi/assembly/libraries.cgi). Locate the precise position of your desired **DNA** part, and outline it with a fine tipped marker. The plate contains dried DNA, so water needs to be added to re-hydrate it and make it useable. Puncture the foil over the well with a pipette and mix the dried DNA in the well with 10ul of autoclaved distilled water. Once the DNA is mixed with the water, let it sit for 5 minutes, then move it to a newly labeled tube or use it for a **transformation**.

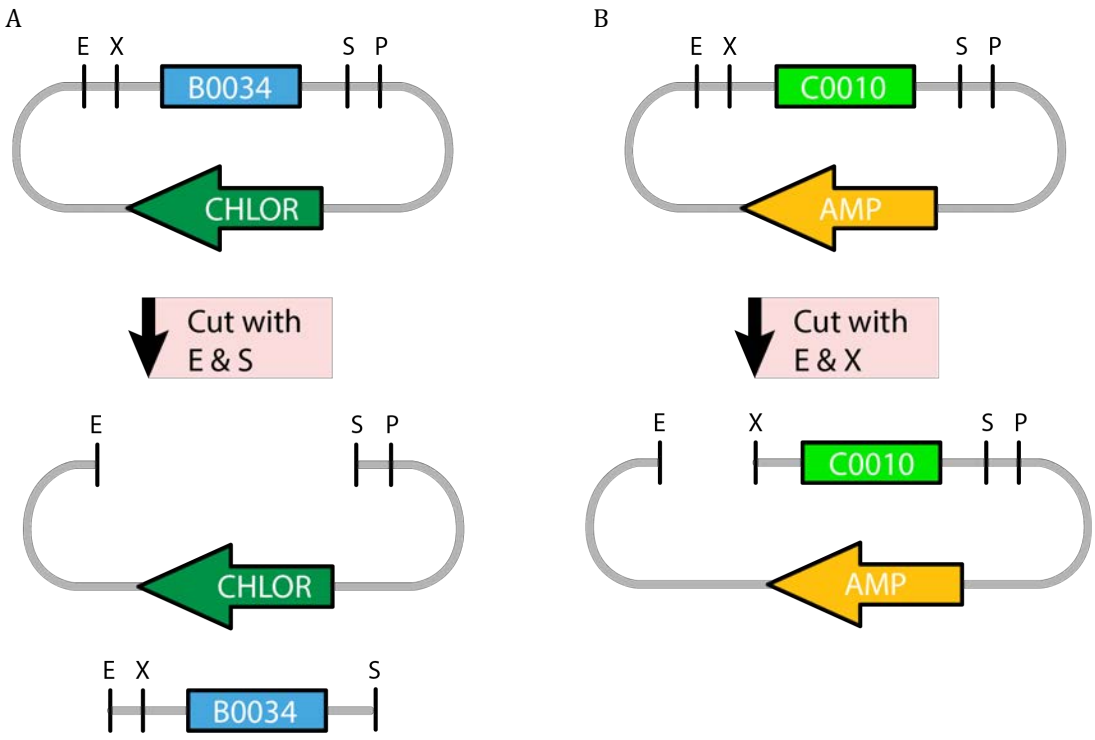
2. Restriction Digestion
See section 3.6 for more background on restriction digestion
See Appendix 1.2 for protocol

A restriction digestion is the process of cutting DNA at specific sequences with molecular scissors. These scissors are known as **restriction enzymes,** and the specific sequences of DNA that they cut at are called restriction sites, or cut sites. Each restriction enzyme has its own unique restriction site. Within a **BioBrick** plasmid, there are specific restriction sites on either side of the gene of interest (GOI), which can be used to either cut out the GOI from the plasmid (as in the example a in the image

below) or to open up a plasmid to insert a different gene into the plasmid upstream or downstream of the GOI (as in the example b in the image below, where another gene would be inserted upstream of part C0010).

The restriction sites located within the BioBrick plasmid are:

- *EcoRI (E)*
- *XbaI (X)*
- *SpeI (S)*
- *PstI (P)*



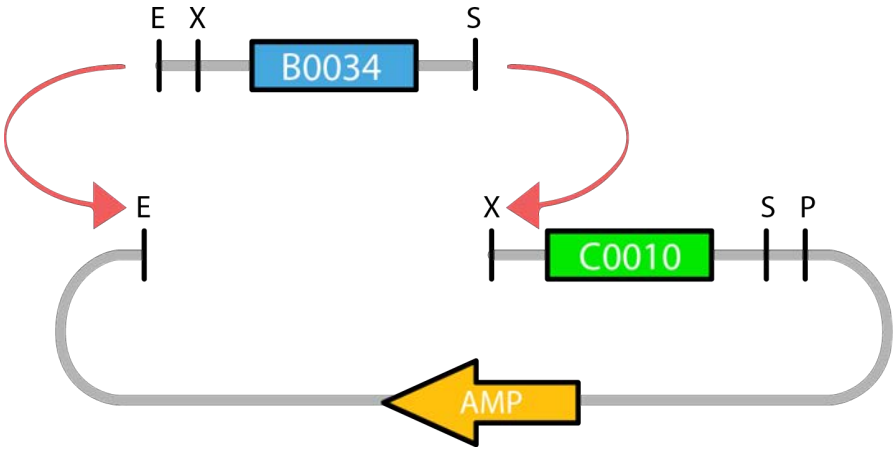
Here, we used the restriction enzymes EcoRI and SpeI to cut one plasmid (left) and EcoRI and XbaI to cut the other (right).

There are two restriction sites on either side of the GOI, and **it makes a difference which ones you use.** That is because in the next step we want to "glue" the GOIs together (or "glue" them into another plasmid) in a process known as a **ligation**. However, only specific sites that have been cut – also referred to as "**sticky ends**"— can be ligated together.

Restrictions sites that can be glued—or ligated—together are as follows;

- E and E
- X and X
- S and S
- P and P
- S and X

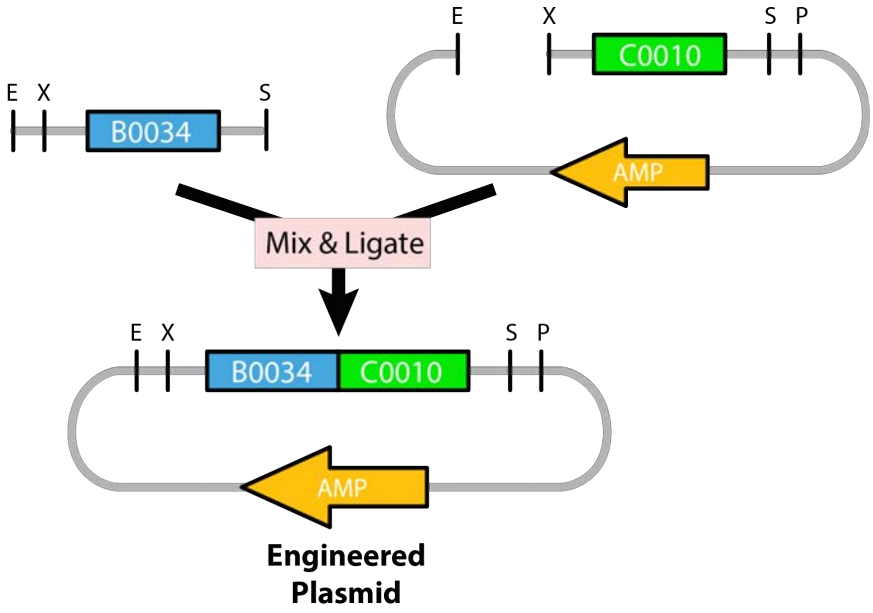
All restriction sites can be ligated back together with themselves, such as the case with EcoRI in the figure below. Additionally, SpeI and XbaI can be ligated together.



Additionally, when you are just opening up a plasmid to put another DNA part in, it is important to cut at the proper restriction sites depending on whether you want to ligate a part upstream (before the GIO in the plasmid, such as a **promoter**) or downstream (behind the GIO in the plasmid, such as a **transcription** stop site).

3. Ligation
See Appendix 1.3 for protocol

A ligation is the process of "gluing" two DNA parts together using the enzyme **DNA ligase**. In order to do this, we need to have linear pieces of DNA (usually from a restriction or PCR) and both DNA parts need to have compatible restriction sites (for more details on compatible sticky ends, see guidebook section 3.6). The "insert" refers to the piece of DNA that is being placed into the "vector". The vector is the part containing the plasmid.

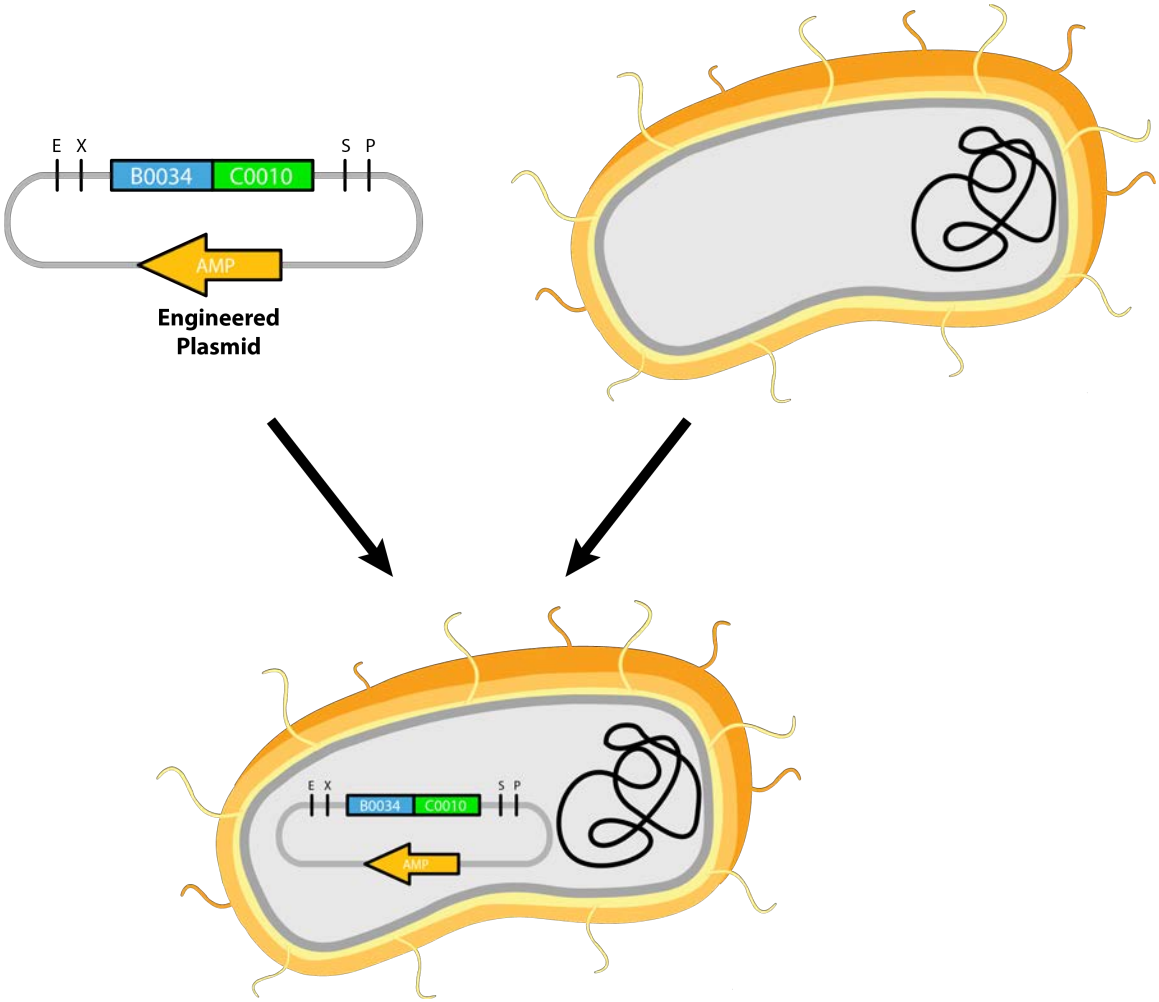


Note that the new piece of DNA now has the exact same restriction sites on either side as the original, separate parts! So, you can now use this same method to add even more parts to your BioBrick.

The space between the two parts, where the S and X sticky ends have ligated together, forms what is called a scar sequence. No restriction enzyme can cut these parts anymore, because the DNA sequence is now a mixture of the X and S sequences, and so can't be recognized by any restriction enzymes (see guidebook section 4.3 for a more detailed explanation of the scar sequence, and some problems with it when it comes to ligating two coding **proteins** together).

4. Transformation
See Appendix 1.4 for protocol

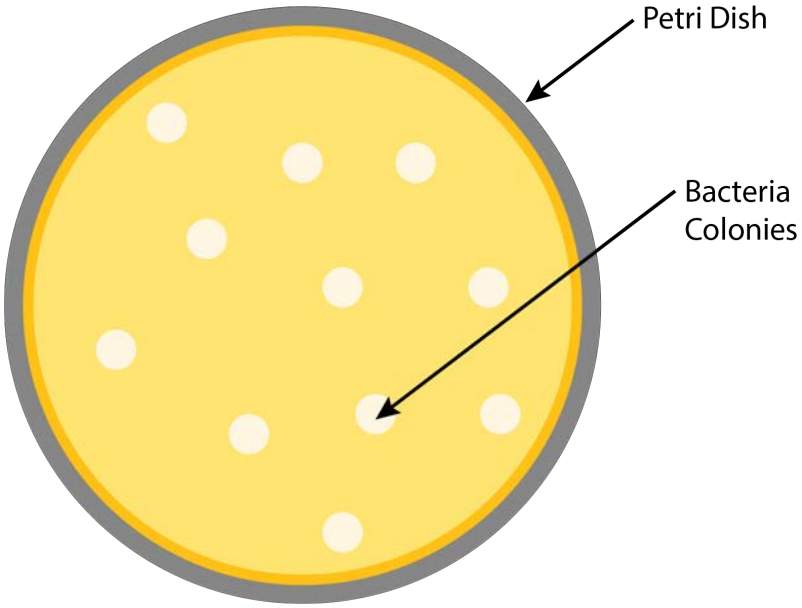
Following ligation, we have a full plasmid with both of our parts together in it. However, we need to be able to isolate plasmids and make copies of them. In order to do this, we transfer the plasmid into bacteria, usually *E. coli*. We get the plasmid into the bacteria through a process called **transformation**.



Bacterial **cells** such as E. coli are surrounded by a lipid membrane and a cell wall. In order to get the **plasmid DNA** into the cells, we must use competent cells for a transformation. Competent cells are

altered so that foreign DNA like our plasmid can get into the cell more easily. Following plasmid entry, the natural DNA replication machinery of the cell replicates the plasmid DNA (for more information on DNA replication, see guidebook section 2.2).

In the figure above, the black line inside of the cell is the genomic DNA, which is different than the plasmid DNA that we insert into the cell. In order to determine which cells have our plasmid inside of them, we utilize the **antibiotic resistance** found on the plasmid (for more detailed information on antibiotic resistance in plasmids, see guidebook section 3.2). To do this, we spread our cells on petri dishes (commonly referred to as plates) that contain agar and a growth medium (nutrients for the cells to grow) as well as an antibiotic, which kills bacteria except those with antibiotic resistance. In the above figure, the plasmid contains the resistance for the antibiotic AMP, or ampicillin. So, if we grow our cells on a growth medium containing ampicillin, all cells that don't contain our inserted plasmid will die. Therefore, the cell colonies that grow on this plate (after an incubation overnight in order to give the cells time to replicate) should contain our plasmid.

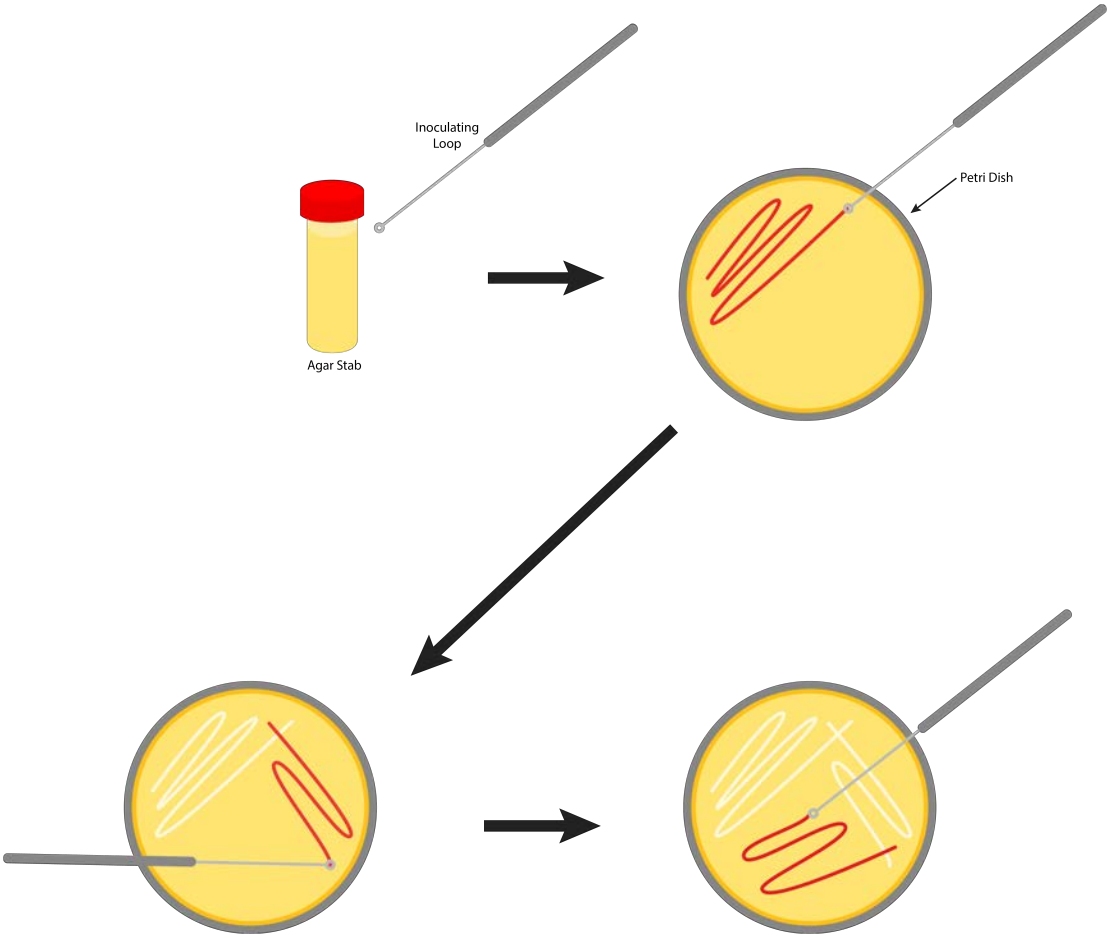


The above plate contains the antibiotic ampicillin, so all of the bacterial colonies (shown in white) growing should contain our plasmid. All of the cells from our transformation that did not have our plasmid would die due to the ampicillin. Once colonies are present on your plate, it's time to grow up single colonies. For this, see methods section 6 below.

5. Growing Bacteria from Agar Stabs onto Plates
See Appendix 1.5 for protocol

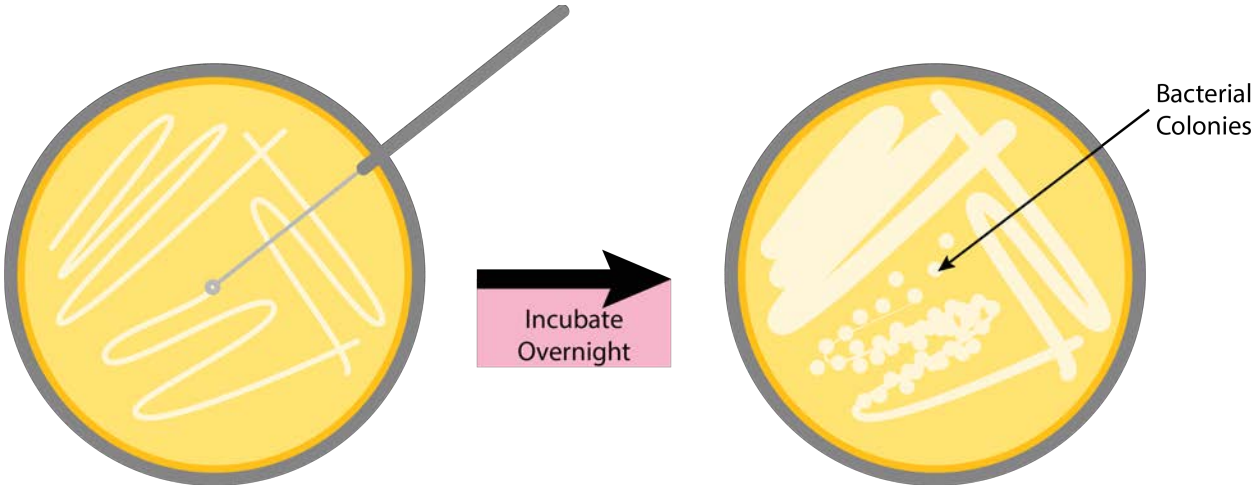
Sometimes bacteria is stored or shipped in an agar stab, which contains LB agar and the appropriate antibiotic for the plasmid the bacteria in the stab carry, as well as bacteria containing a plasmid of interest. In order for you to use the bacteria from an agar stab, the culture needs to be plated, and a single bacterial colony needs to be recovered.

The method for plating the culture involves using an inoculating loop to "streak", or **inoculate**, a plate (see figure below). The point of streaking a plate is to dilute the amount of bacteria, so that single colonies become visible after overnight incubation.



First, a sterile inoculation loop or sterile pipette tip is put into the agar stab, and then it is used to create the first streak by moving the loop across the agar in a zig-zag motion in one quadrant of the plate (see above figure). There will be lots of bacteria on the inoculation loop at this point, and therefore it will likely be difficult to pick out a single colony once the plate has been incubated overnight and the bacteria has had a chance to replicate. Because of this, we re-sterilize the inoculation loop by passing it through the flame of a Bunsen burner several times (then wait several seconds for it to cool off), and re-streak the plate by passing the inoculation loop once through the end of the previous streak, and then use the same zig-zag motion to streak a second area of the plate. Re-streak the plate once more (following the same method as before), and incubate it overnight.

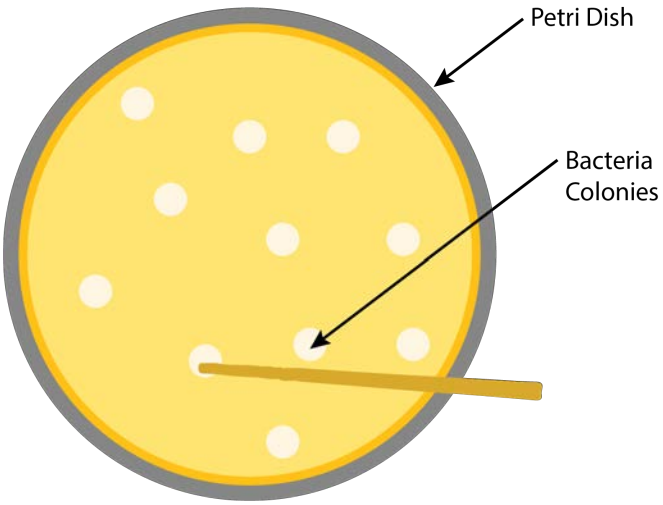
Following incubation, you should be able to see single colonies! See the next section (5.6) of this chapter on how to grow up single bacterial colonies .



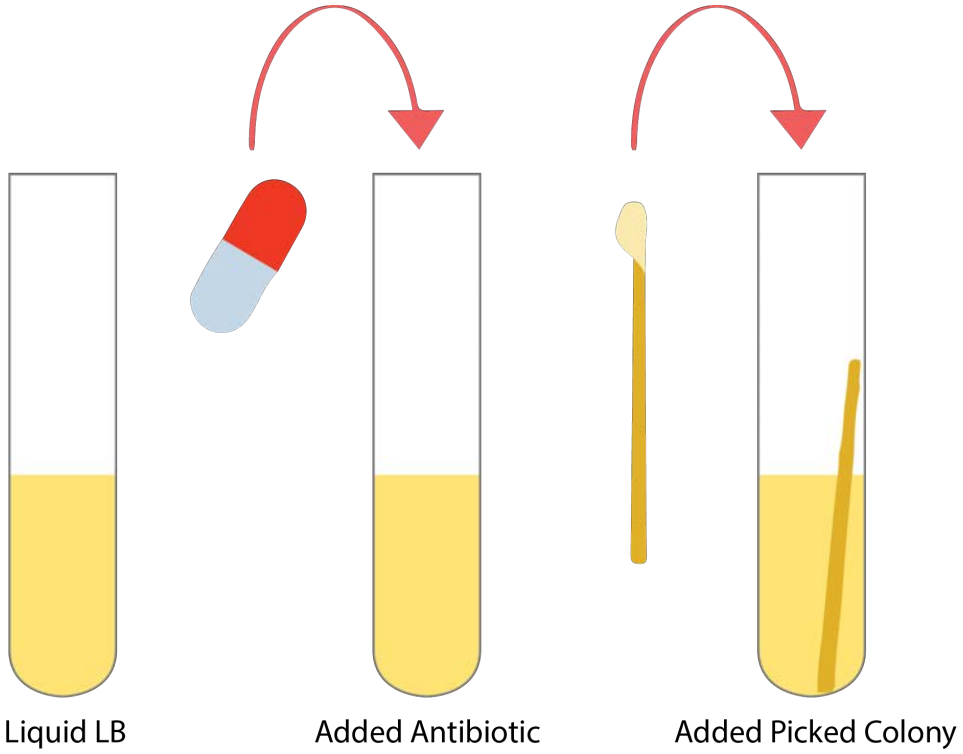
6. Growing up Single Bacterial Colonies
See Appendix 1.6 for protocol

Each separate colony on the plate started with a single bacterial cell that was successfully transformed with a plasmid from our ligation, or from plating our agar stabs. This cell then replicated to produce visible bacterial colonies on the plate. In order to see if that plasmid has our successfully assembled part, we need to grow some of these colonies in liquid media in order to isolate the plasmid DNA (**miniprep**).

To grow up these colonies, we label them on the plates and label tubes in the same way. Make sure to number individual colonies and mark the corresponding number on the tube, this way we know which liquid cultures came from which colonies on the plate. To make sure we are only growing cells that contain a plasmid that might be our final assembled part, we put the same antibiotic that was on the plate into a test tube with liquid LB media. (Note: once colonies are picked, place the plate in the fridge at +4°C, agar side up and lid side down, for later use (ex. for making glycerol stocks or agar stabs).



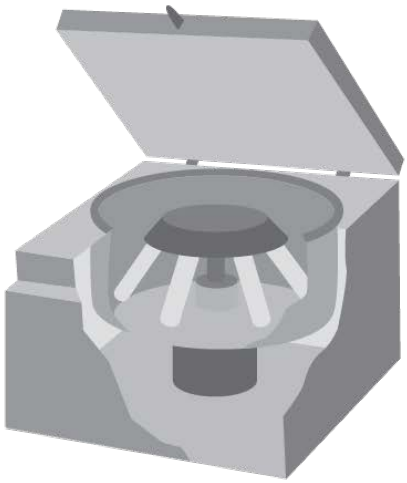
Once you have the colony picked by gently poking it, you can put the sterile toothpick or pipette tip into a tube containing liquid LB media and the same antibiotic that was on the plate. After leaving the tube in the shaking incubator over night (about 14 hours), you will have enough cells in the liquid media to isolate and purify the plasmid DNA. You should pick a few colonies (put each colony in a separate LB tube) to make sure that you pick at least one containing copies of the plasmid you wanted to make.



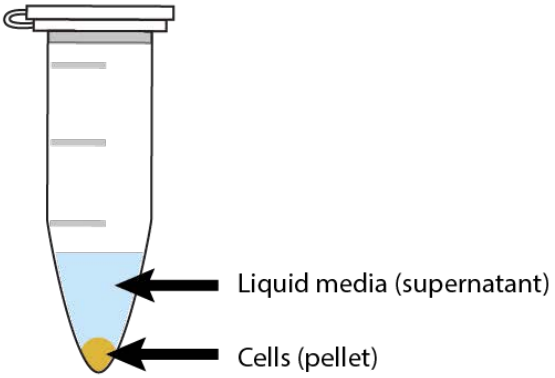
7. Mini-prepping – Purifying Plasmid DNA
See Appendix 1.7 for protocol

Once our cells from the plate colonies have grown overnight in liquid containing antibiotic, it is time to miniprep – or purify—our plasmid DNA. When the cells grow they will replicate, so we will have lots of cells, and therefore lots of DNA.

After we have grown our cells we get rid of the growth medium that we grew them overnight in, and we do this through centrifugation. This step basically spins the tubes very fast, so our cells sink to the bottom, forming a cell pellet, and we can then get rid of the liquid growth medium either by **pipetting** the liquid medium out (being careful not to touch the cell pellet), or by just pouring it out of the tube.



Centrifuge



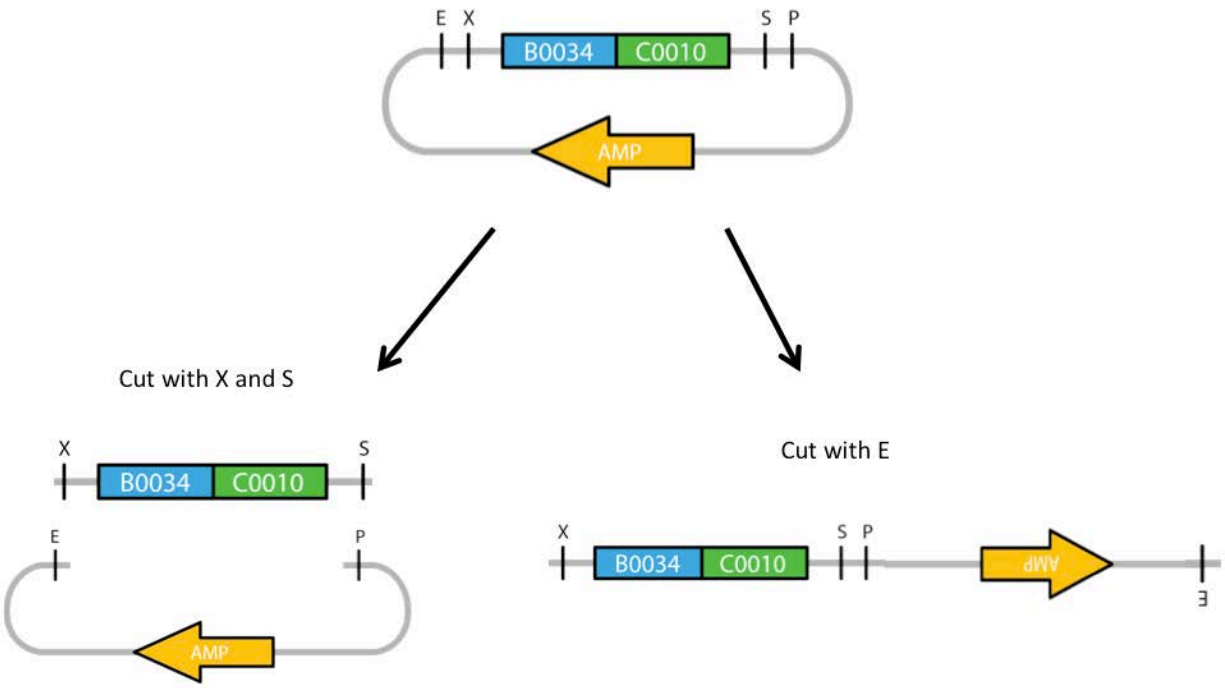
After this, we then lyse cells, or break the membrane, in order to release our DNA. However, there are many different components inside the cells, so when we lyse them, it will also release other (genomic) DNA, RNA, proteins and everything else inside the cell. The thing we want to do, then, is separate our plasmid DNA from everything else.

To do this we add reagents that contain RNase (which is an enzyme that breaks up RNA) as well as reagents that **denature** the DNA and proteins. We then add solutions to re-nature the cellular components, but only the plasmids are able to re-nature, due to their circular shape, small size and supercoiled configuration. Instead of re-naturing, the genomic DNA and proteins form a precipitate. When we then **centrifuge** the solution, all of the precipitated components fall to the bottom.

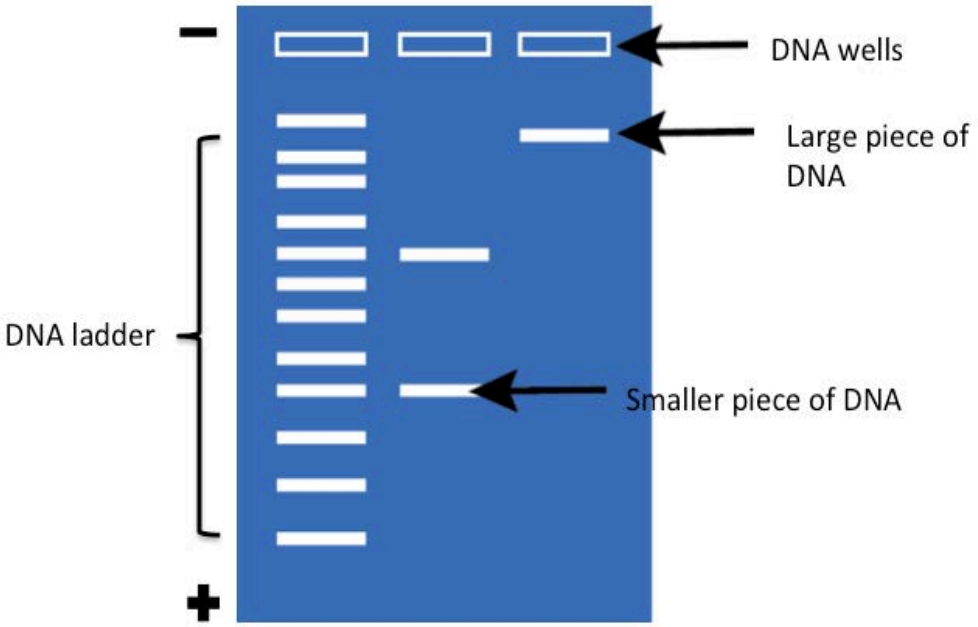
After we centrifuge this solution, the **supernatant** (the liquid at the top) contains our plasmid DNA (since the precipitated proteins and genomic DNA have been forced to the bottom through centrifugation). We can then put this liquid into a spin column, and this "grabs on" to our plasmid DNA and lets everything else flow through. Following this, we wash the column to get rid of any residual nucleic acids and genomic DNA. We can then put the column into a fresh tube, and "release" the plasmid DNA by adding a different solution (called elution buffer). Now, we have a tube that contains only our plasmid DNA in a **buffer**.

8. Running Agarose Gel Electrophoresis
See Appendix 1.8 for protocol

Following a miniprep or PCR, or any other circumstance in which you need to know the size of your DNA part, an agarose gel should be run in order to confirm the size. If you are trying to find out the size of a plasmid, the DNA should be restricted with a single restriction enzyme in order to linearize it (make it a string of DNA as opposed to a circle of DNA). However, if you want to find out the size of your GOI without the plasmid you can cut one restriction site on either side of the GOI (see image below; also see guidebook section 5.2 for more details on restriction).



Gel **electrophoresis** takes advantage of the fact that DNA is negatively charged, so by running a current through the gel, the DNA will migrate towards the positively charged end. Because smaller pieces of DNA can travel faster through the gel, it will separate the different sized strands of DNA according to their size, with larger ones closer to the wells (negatively charged end), and smaller ones closer to the end of the gel (positively charged end). A DNA ladder is used to compare the size of your DNA with known sizes of DNA.



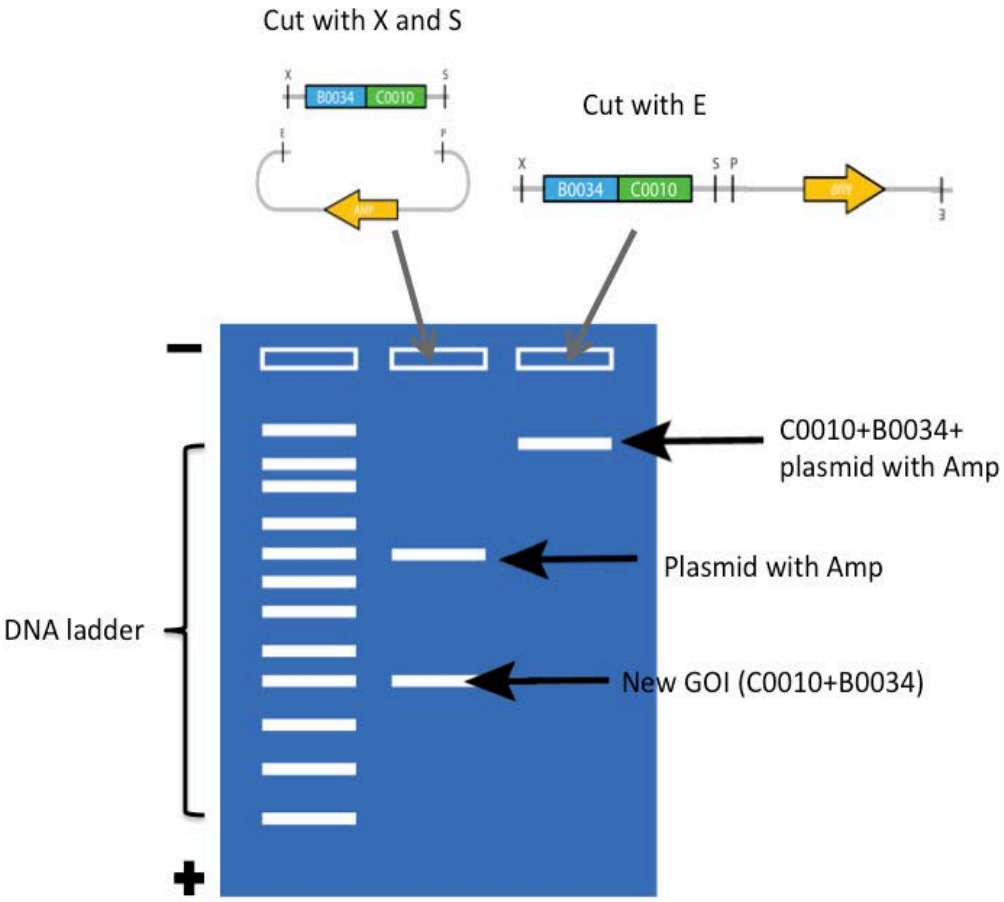
To run a DNA gel, the agarose gel is made with the appropriate concentration of agarose (see table below) and let to set with a comb embedded, making wells into which the DNA will be later pipetted. The gel is then covered with a buffer. The DNA is mixed with distilled water and loading buffer. The loading buffer contains dyes (such as bromophenol blue) to help determine the approximate position of the DNA in the gel as it's running. Loading buffer also contains a reagent to increase the density of your DNA loading solution, so your DNA will not float out of the wells as easily. The DNA solution is put into the wells using a pipette to carefully—and slowly—load the solution containing the DNA and loading buffer. Don't forget to add a ladder, and make sure you record which well contains the ladder and which contains your DNA sample! The volume loaded depends on the size of the wells and the concentration of your DNA.

Linear DNA Resolution for Gel Electrophoresis

% Agarose Concentration (w/v)	DNA size (bp)
0.5	1,000 – 30,000
0.7	800 – 12,000
1.0	500 – 10,000
1.2	400 - 7000
1.5	200 – 3,000
2.0	50 – 2,000

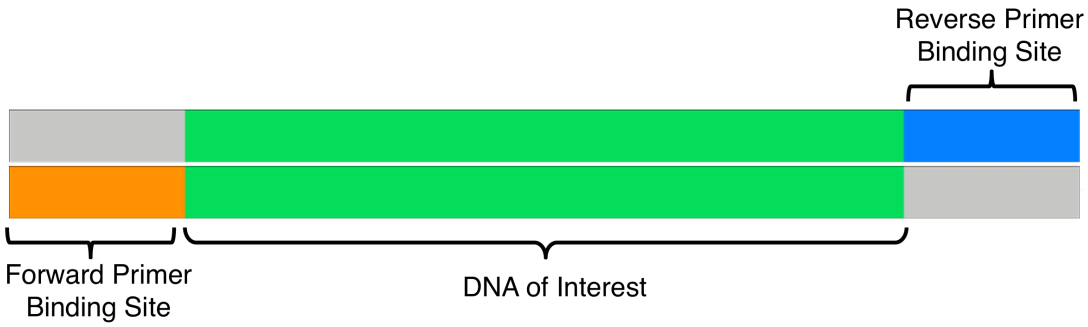
The gel apparatus is then hooked up to the power supply and you should gradually see the dye in the loading buffer move towards the positive end of the gel. After your gel is done running, the DNA is visualized with a dye that binds to DNA and can be seen under a transilluminator.

The size should be compared to a standard ladder. The size of the new part should be the added size of each individual part. If we cut at restriction sites on either side of the new GOI (for example, X and S), we would add the **base pairs** of the DNA parts, not including the plasmid. However, if we just linearized the plasmid with one restriction enzyme (say, E), we would add the base pairs of the parts, plus the base pairs of the **plasmid backbone**. Expected sizes can be looked up on the registry of standard parts (see guidebook section 6 for more details on the registry). These base pair sizes can then be compared to the known sizes of the DNA ladder parts, and thus you can confirm whether or not your part has been successfully made.



9. PCR
See Appendix 1.9 for protocol

PCR stands for **Polymerase Chain Reaction**. Polymerase, because it uses the enzyme **DNA polymerase** to make single stranded DNA into double stranded DNA; Chain Reaction, because each cycle of a PCR makes the starting components for the next cycle. PCR is very useful for making many copies of a piece of DNA that is between regions that have a known sequence. These known sequences are primer binding sites, which are complementary to small single stranded DNA molecules called **primers**. These primers, when bound to the primer binding sites, give the DNA polymerase a place to start replicating the DNA you are interested in.

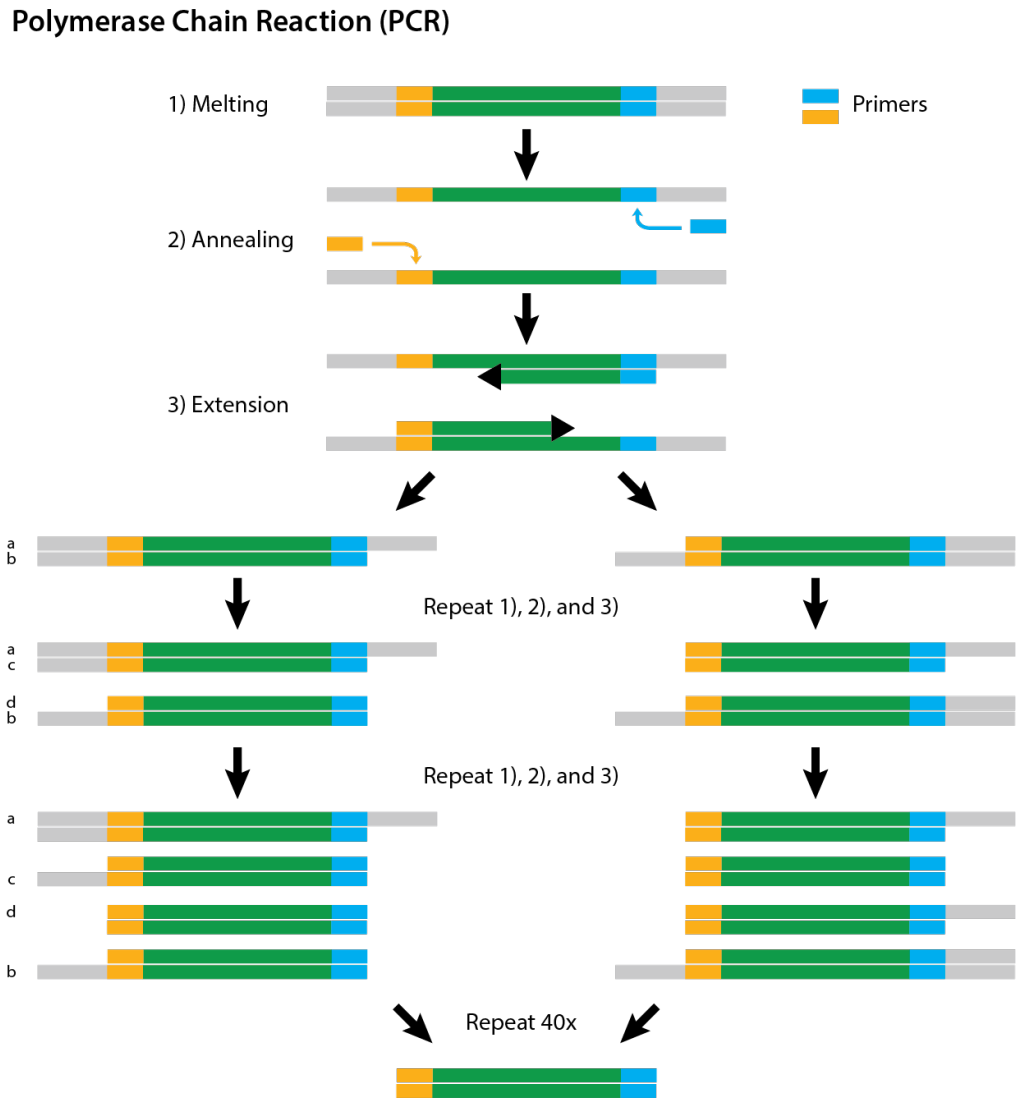


PCR has 3 unique steps in each cycle of the chain reaction:

1) Melting – Here the double stranded DNA in the reaction mixture is separated into single stranded DNA molecules by heating up the tube that the reaction mixture is in.

2) **Annealing** – Annealing is the process of two pieces of single stranded DNA that are complementary binding together. In PCR, annealing happens between the primers and the primer binding sites. This gives DNA polymerase a place to start making more double stranded DNA. The temperature must be lowered for annealing to happen.

3) Extension – This is the step where the temperature of the reaction tube is just right for DNA polymerase to extend the short primer and turn the single stranded DNA template into a double stranded molecule.



Exponential increase of copies of sequence between primer sites

10. Making Glycerol Stocks
See Appendix 1.10 for protocol

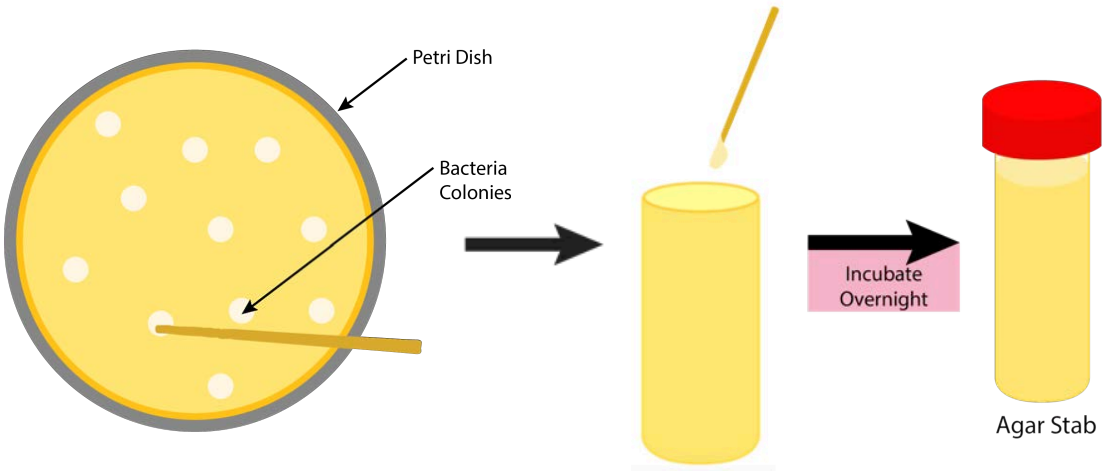
After confirming that a part has been assembled successfully, it's important to save the part in order for it to be accessible for later experiments. The part can be saved as a mini-prep (i.e. just the DNA), in order to be later transformed into new bacteria. However, it's always a good idea to also save bacteria containing the plasmid DNA as well. One way of saving bacteria with your plasmid for long-term use is by making glycerol stocks. This method requires a -80 °C freezer—for a method that does not require a -80 °C freezer, see the next section in this chapter (5.11).

After determining which colonies have your plasmid, choose one colony that has the successfully assembled part and grow it in liquid LB with the appropriate antibiotic overnight (see section 6 of this chapter for more details on picking colonies). After that, mix the cells carefully with glycerol in a tube, and then place the tube in the -80 °C freezer. Glycerol protects the cells from damage caused by freezing, so if we need more of the cells (in order to get more DNA), we can just take cells from the glycerol stock and they can be used again!

11. Making Agar Stabs
See Appendix 1.11 for protocol

In addition to glycerol stocks (see section 10 of this chapter), one way of preserving a plasmid in bacteria is by making an agar stab. Although bacteria doesn't last as long in an agar stab as it does in glycerol stocks, this method can still be used to preserve bacteria for up to two years in the fridge (4°C).

Once you have your plasmid of interest, choose one colony that has the successfully assembled part. A toothpick or pipette is then used to get bacteria from the colony and stab it in an agar tube with the appropriate antibiotic. The tube is then incubated overnight and stored in the fridge. See section 5 of this chapter and guidebook appendix 1.5 on how to recover bacteria from an agar stab.



12. DNA Synthesis

Design of Synthesized Constructs

Many iGEM teams looking to submit new parts to the registry consider DNA synthesis as a strategy for assembling these parts. Although the cost and time required to synthesize large parts (over 1 kb) can be prohibitive, synthesizing small parts can sometimes save you both time and money. Some teams opt to synthesize entire constructs so that they can begin testing immediately while other teams synthesize sections of their constructs in order to simplify their construction strategies. For example, teams may have a promoter and **ribosome binding site** synthesized with the BioBrick cut sites on either side so that they only have to do one construction to add an additional gene. Reducing your required constructions from two to one could mean the difference between finishing your construct and not finishing it. Whatever you decide to order, you will still need to put it into a BioBrick vector to submit to the registry. This can be accomplished by making sure to synthesize your construct with the BioBrick **prefix** and **suffix** and then cutting with a combination of two enzymes with cut sites on either side of the part. A BioBrick vector would also be cut with the same enzyme combination and the two pieces ligated together.

If your team decided they would like to synthesize on or more parts, there are several options for synthesis companies. Common choices are IDT, BioBasic and Genscript. Each company offers slightly different rates and turnaround times for orders so it can be useful to price out your order using all three to see which would be the best, keeping in mind of course that you be limited on time and need to go with the fastest option. Some of these companies also sponsor iGEM teams from time to time, so make sure you check out the iGEM website (igem.org) or contact them directly to see if there might be a discount available.

Once you have selected a synthesis company, there are a few things you will need to think about when placing your order. First, you will need the sequence of the part you want to order. Generally, you only need to know the forward sequence even though they will sequence it for you double stranded. Make sure you remember to include sequences for any additional features you desire such as scar sequences and cut sites. For example, if you wanted to have a promoter and ribosome binding site synthesized, your final sequence that you order would include BioBrick prefix followed by the promoter sequence, then the scar sequence, followed by the ribosome binding sequence, followed by the BioBrick suffix. You would order this entire string of DNA together as one sequence.

It is also important to carefully consider the vectors that they offer your part in. Often companies will send your part in a common vector such as puc19. It is important to pay attention to how this might affect your assembly strategy. If they put the part in a pUC19 plasmid for example, which has an *XbaI* site in the vector, you will not be able to cut your construct out of the vector using *XbaI* in order to move it into a BioBrick vector. For this reason, it is important to check the cut sites in the vector. Often they will offer a 'simple vector' choice that will have the majority of the common cut sites removed so that you do not run into this problem. It is very important to look for this when you are placing your order as this is often the vector you want to choose. Some companies also allow you to specify which sites not to include in the vector. In this case, you would need to be sure to specify the six BioBrick cut sites (*EcoRI, NotI, XbaI, SpeI, PstI*) You also need to pay attention to the **antibiotic resistance** of the vector they send you. Most often, they will send you an amp resistant vector. This means you will need to grow cells containing this vector in ampicillin. Generally you want to avoid chloramphenicol resistant vectors as this will make it difficult to move your construct into a BioBrick chloramphenicol vector with proper selective pressure.

The final thing you will need to watch out for is the difference between gene fragments and constructs in vectors. Some companies will offer 'gene fragments' (at IDT they are called gBlocks). These are fragments of linear, double stranded DNA. These parts do not come in vectors and as such cannot be used the same way. These are often used for Gibson Assembly and require additional design strategies. If this is something you are interested, it is best to ask someone for help in designing these.

Using synthesized constructs

When you receive your synthesized DNA construct, there are several things that need to be done. Often the DNA is shipped in a dried format and needs to be rehydrated. Read the instructions provided very carefully as they will walk you through this process.

Once rehydrated, you generally need to transform the construct into competent cells. This is important as they often only give you a very small amount of the DNA and you need to make more to be able to work with it. Again, make sure to follow the protocol for this, paying careful attention to the concentration of DNA that should be added to the competent cells. Remember, more DNA is not always better! After transformation, overnight cultures can be made and plasmid DNA extracted. You may then like to verify that you have the right construct through restriction digestion and gel electrophoresis and/or DNA sequencing. Note that to sequence your construct in the vector provided, you will need specific primers for this. You may simply want to make sure you have the right size construct using digestion and gel electrophoresis and sequence later on. The next step would be to plasmid switch the construct into a BioBrick vector. Again, this can be accomplished by cutting both your construct DNA and a BioBrick vector with a combination of two enzymes with cut sites on either side of the part. These two pieces are then ligated together and regular cloning steps are followed (transformation, screening, cultures, extracting DNA, etc). Once you have plasmid DNA of your part in a BioBrick vector, you may wish to send this for DNA sequencing using BioBrick primers. Once the sequence is confirmed, you are ready to test or begin assembling it with another part!

6. THE REGISTRY

Lisa Oberding and Emily Hicks

1. The Registry of Standard Biological Parts

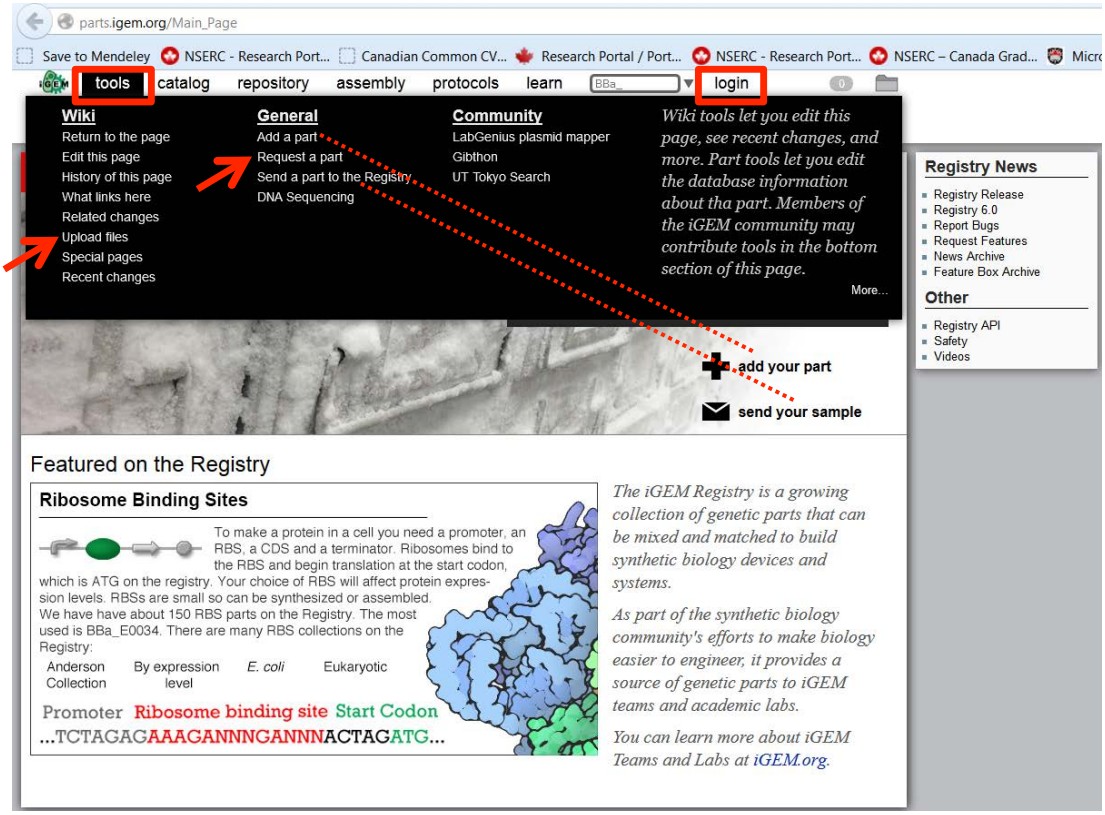
With iGEM teams all over the world creating and testing **Biobricks**, there needs to be some kind of repository for both the **DNA** parts and all the data about them. One of the key contributions that iGEM has made to the **synthetic biology** community has been to build such a repository - the Registry of Standard Biological Parts, often abbreviated as 'the registry'. Having both a physical and a web entity, the registry serves two major purposes: to store physical DNA for Biobricks and to communicate information about these Biobricks in an open-source setting. Located in Cambridge, Massachusetts is the physical repository stores all the DNA parts created. iGEM teams and other labs send in their BioBricks as **plasmid DNA**, which gets stored on site. iGEM Headquarters spends time trying to ensure quality and accuracy of the documented parts. Each year, iGEM teams receive a portion of the registry for use in their own projects. Teams use these parts to build their own new constructs, contributing novel parts in the process which they add to registry. They share the data they collected on the new constructs using the parts registry website, which we will cover in more detail in the next section. This idea of sharing and building upon previous teams work is a cornerstone of the iGEM competition.

2. How is the registry organized?

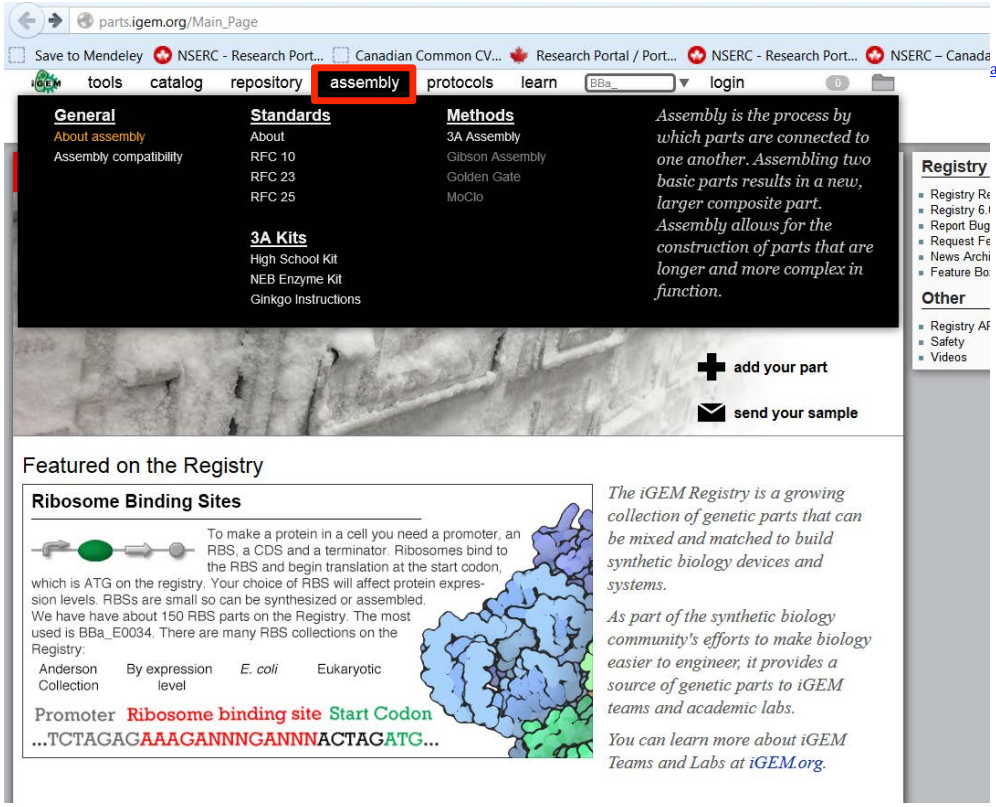
In the registry, each individual Biobrick 'part', whether it is a simple **promoter**, or a composite biobrick containing a variety of subunits, has its own part page. As we will explain shortly, each part page contains different types of information, for example what its subunits are if it is a composite part, or where the DNA came from if it's a basic part. To make things easier, the registry has assigned a variety of symbols to each 'type' of part. Some examples of categories are: promoter, **terminator**, **ribosome binding site**, etc. These symbols are similar to those used in the Synthetic Biology Open Language (SBOL) Visual, which is an open-source graphical way of representing parts (http://www.sbolstandard.org/visual).

3. How do you search the registry?

Although the registry is a fantastic resource, sometimes it can sometimes be difficult finding what you want. To make sure that you are not 're-inventing the wheel', it is important to do a thorough search through the registry prior to building a new part from scratch. If they don't do this, teams may spend large amounts of time building a part only to discover that it had been submitted by a previous team and had been in the registry all along. Below is a guide to to walk you through what is stored in the registry, the basic tools available, as well as some tips to make your searches easier.



To use the registry, you must first log in to your iGEM account, using the link in the top right corner. The tools menu will give you the option to upload files (such as images), add a page for a part you have created, and send parts to the registry (these links are also available directly on the homepage, which will be explained later). You can also use the tools menu to request a part from the registry that was not included in the initial distribution package.



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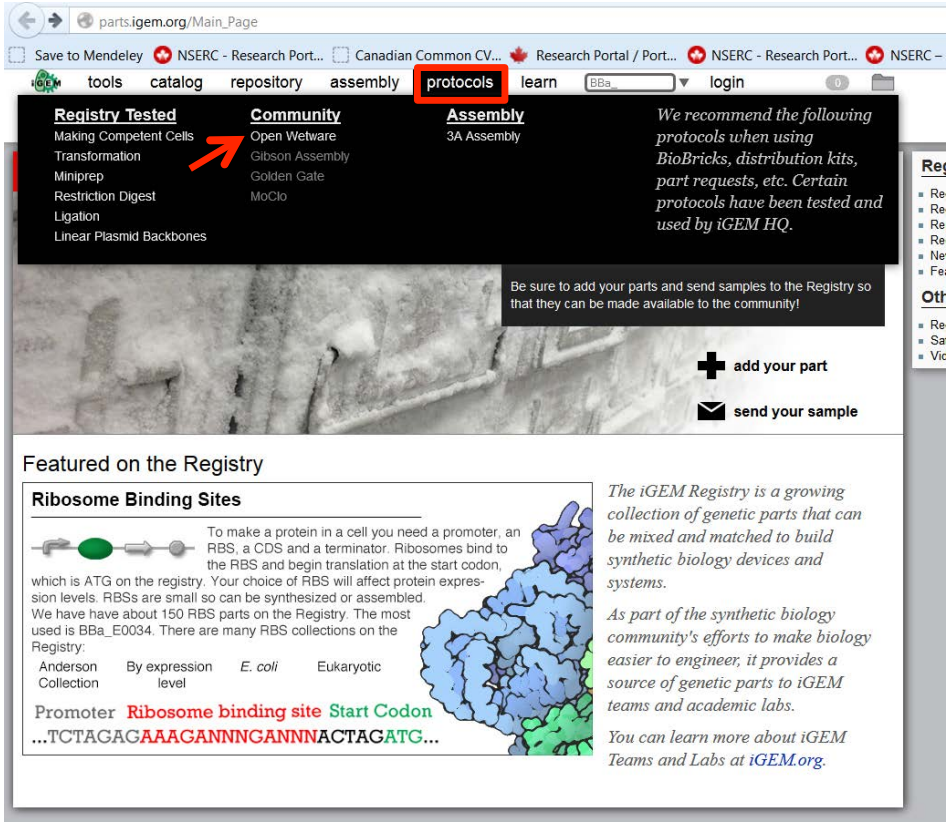
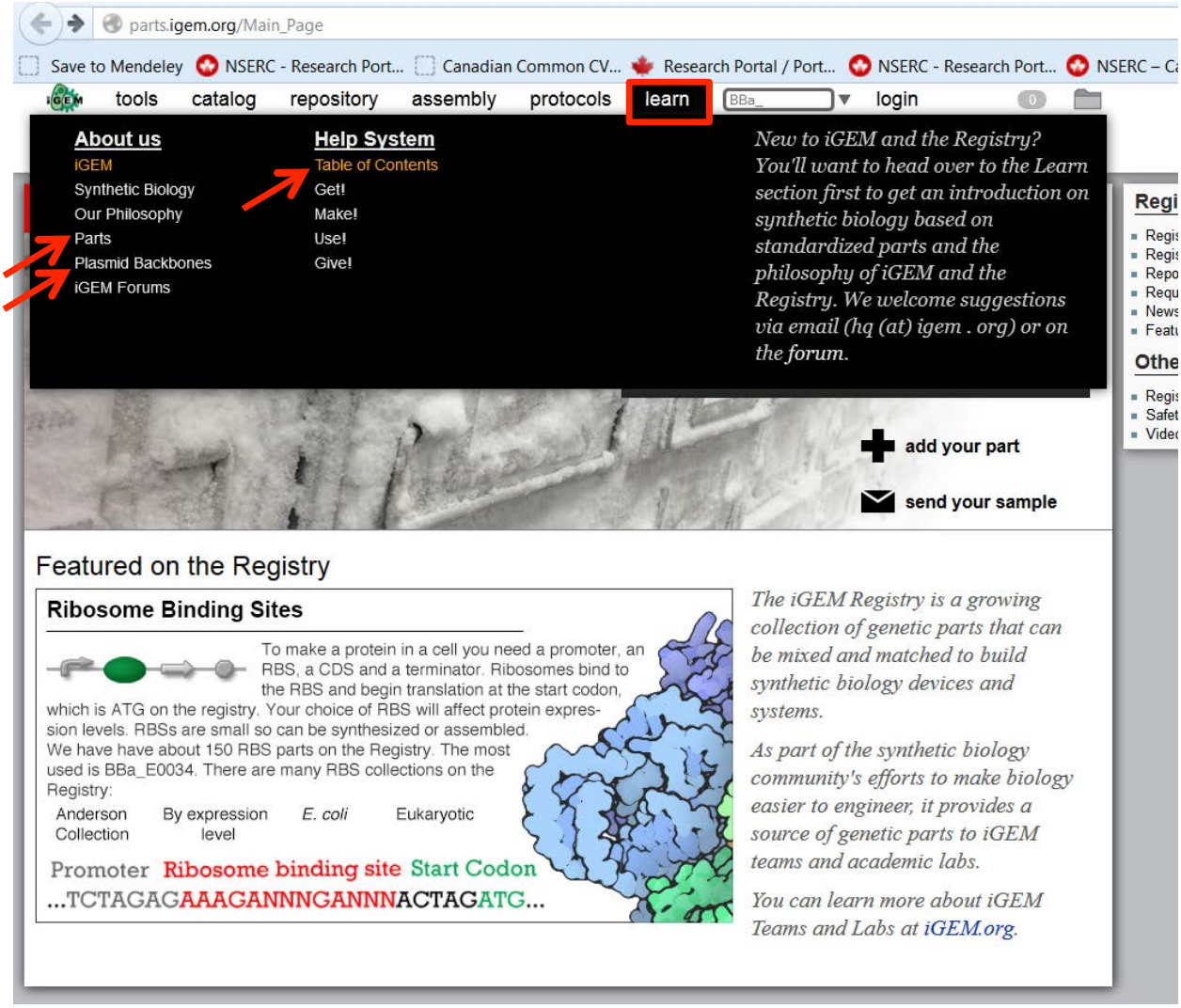
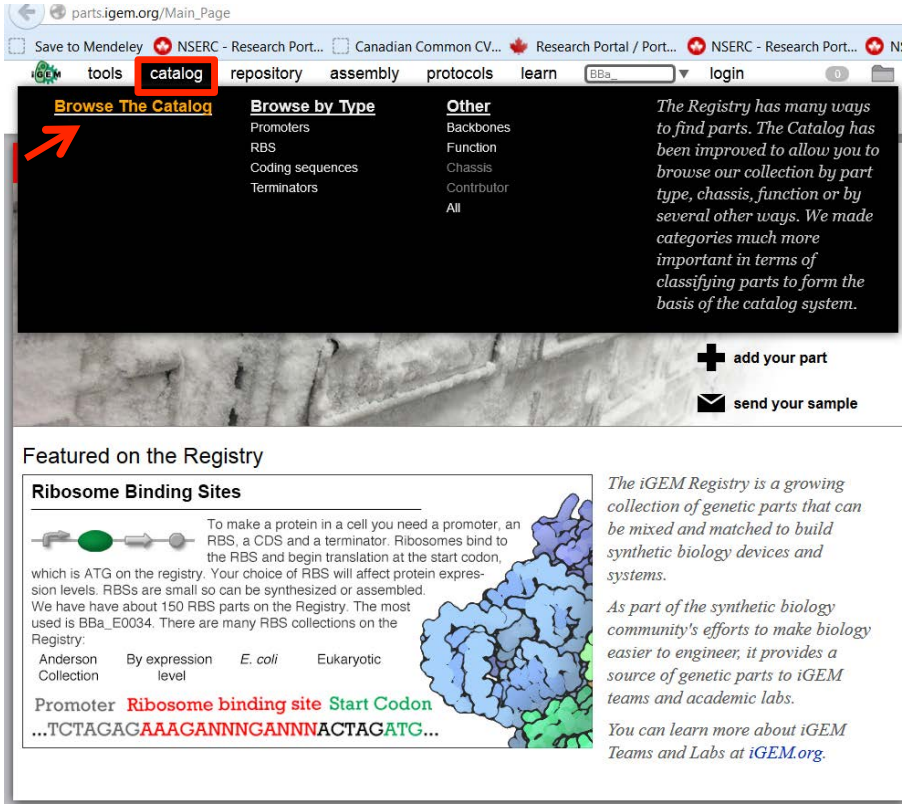
The assembly menu will give you links to information about the standard assembly methods – RFCs - that are used to create BioBricks. Three RFC standards are most commonly used: RFC10, RFC23, and RFC25. Any part submitted to the registry MUST be compatible with RFC10 - which means that the submitted part (1) MUST have the standard BioBrick **prefix** and **suffix** sequences, and (2) the standard cut sites in the prefix and suffix MUST NOT be present anywhere else inside of the part sequence. RFC23 - a standard that is not mandatory- uses a modified prefix and suffix to create a different scar site that can be used to create **protein** fusions. RFC25 is also not mandatory. It includes additional cut sites that can be used to create protein fusions. To learn more about each of the assembly standards, you can click on the links. Here you can also find information on 3A assembly, which is another way of putting two BioBricks together into a different **plasmid backbone**.



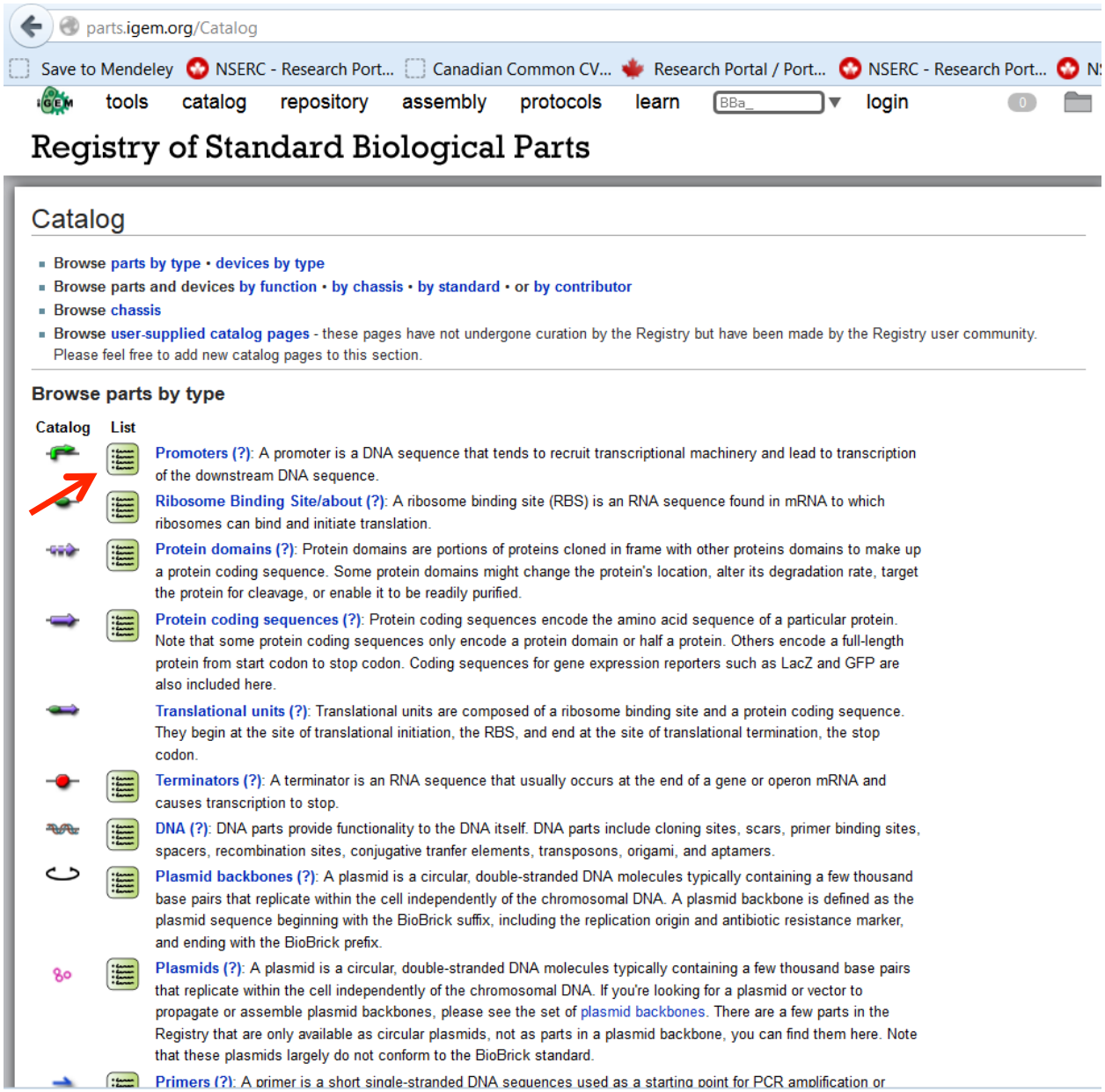
In the “Protocols” section, you can find common lab protocols that you will need during the course of your project. In addition to those listed on the registry and in this guidebook, the registry provides a link to “Open Wetware” - a great online resource where you can find many lab protocols and other useful information about experimental work pertaining to iGEM.



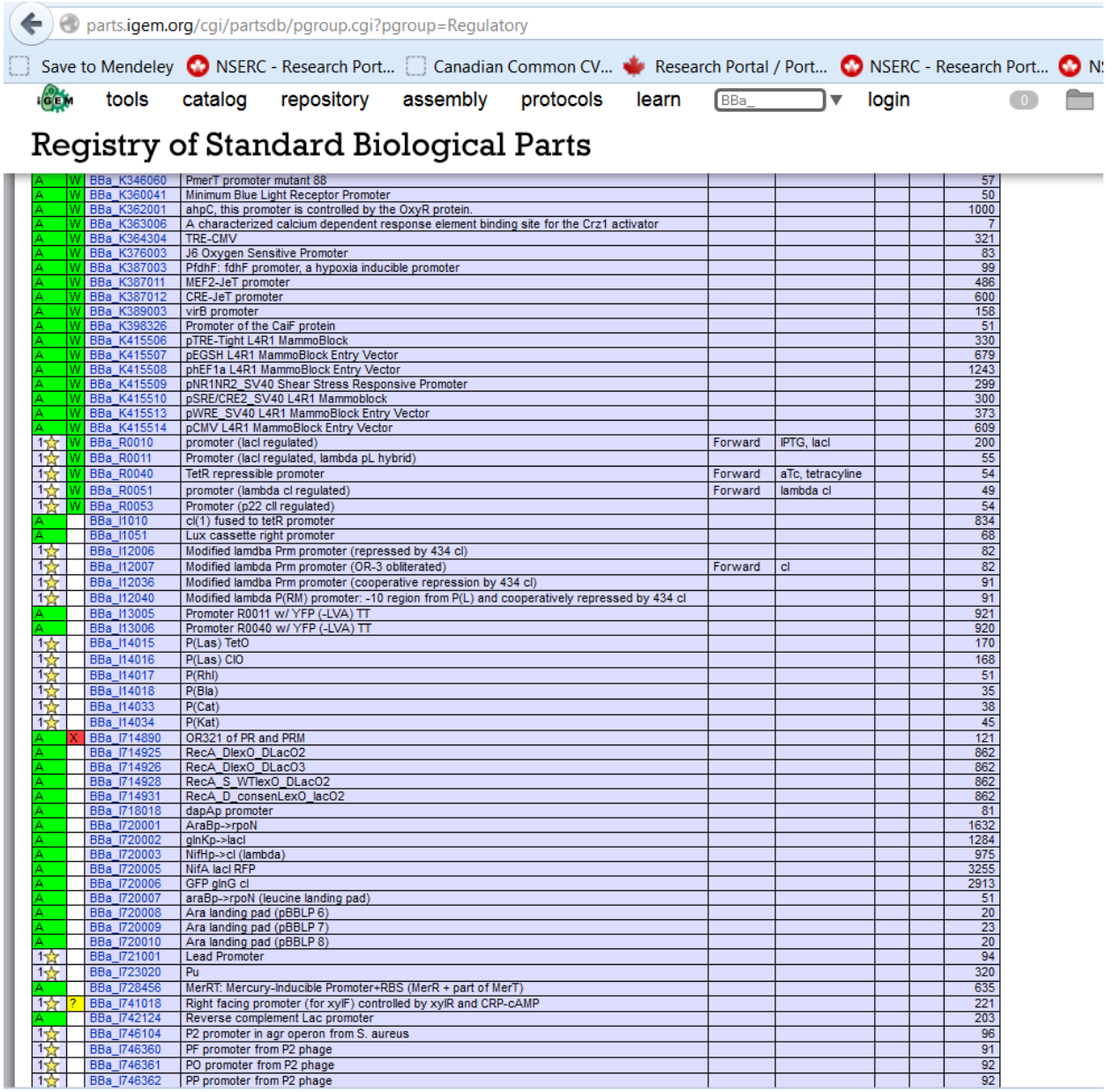
In the "learn" section of the registry menu there are links to lots of information useful to new iGEM teams, as well as information on iGEM itself. Specifically, the "Parts" and "Plasmid Backbones" will give you background information on the BioBrick system you will be using in your project. The "Help System" menu has further information on how to utilize the parts registry beyond what information is given in this guide.



The "Catalog" menu will allow you to browse parts previously submitted to the registry by type. Note that not all parts of a certain category will be listed under the respective section of the catalog; therefore, by running a specific search instead of browsing a category you have a better chance of finding what you are looking for, as some parts may not have been categorized in the catalog yet. Under "Backbones" you will find information on the common plasmids you will be working with to assemble your BioBricks. By clicking "Browse the Catalog", you can find more information on the different categories of DNA parts.



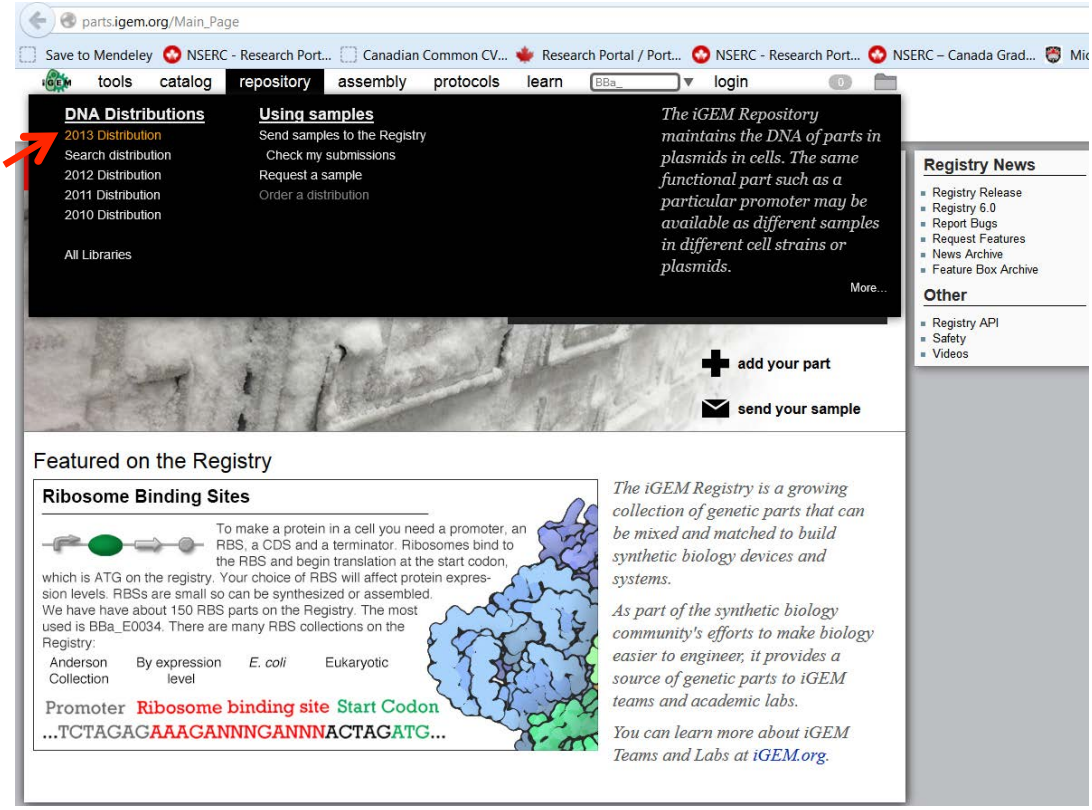
The catalog has parts organized by their function, and tells you what each part is for. It also shows the standard symbol that is used to display that type of part on a parts page based on its function. By clicking the link for a specific category, you will be taken to a page with further information on that type of part and its design, as well as a catalog list of subcategories to help you narrow your search to the exact type of part you need. By clicking on “list” on the main page (above) you will be given a full list of parts of that particular type.



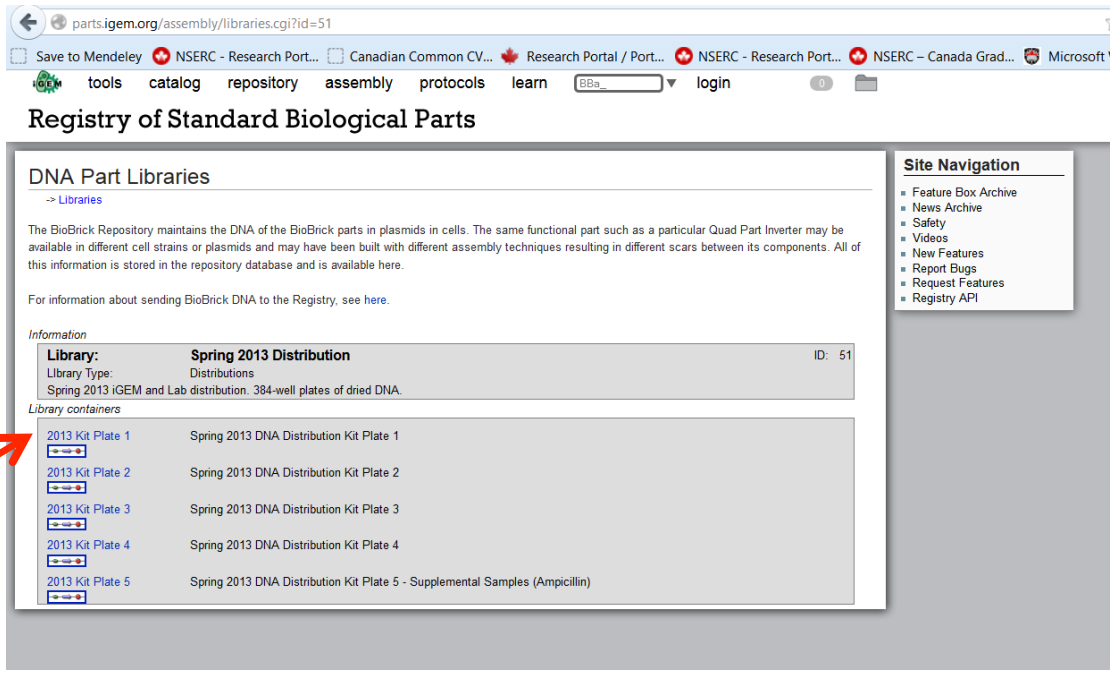
A	W	BBa_K346060	PmerT promoter mutant 88					57
A	W	BBa_K360041	Minimum Blue Light Receptor Promoter					50
A	W	BBa_K362001	ahpC, this promoter is controlled by the OxyR protein.					1000
A	W	BBa_K363006	A characterized calcium dependent response element binding site for the Crz1 activator					7
A	W	BBa_K364304	TRE-CMV					321
A	W	BBa_K376003	J6 Oxygen Sensitive Promoter					83
A	W	BBa_K387003	PfdhF: fdhF promoter, a hypoxia inducible promoter					99
A	W	BBa_K387011	MEF2-JeT promoter					486
A	W	BBa_K387012	CRE-JeT promoter					600
A	W	BBa_K389003	virB promoter					158
A	W	BBa_K398326	Promoter of the CaiF protein					51
A	W	BBa_K415506	pTRE-Tight L4R1 MammoBlock					330
A	W	BBa_K415507	pEGSH L4R1 MammoBlock Entry Vector					679
A	W	BBa_K415508	phEF1a L4R1 MammoBlock Entry Vector					1243
A	W	BBa_K415509	pNR1NR2_SV40 Shear Stress Responsive Promoter					299
A	W	BBa_K415510	pSRE/CRE2_SV40 L4R1 Mammoblock					300
A	W	BBa_K415513	pWRE_SV40 L4R1 MammoBlock Entry Vector					373
A	W	BBa_K415514	pCMV L4R1 MammoBlock Entry Vector					609
1☆	W	BBa_R0010	promoter (lacI regulated)	Forward	IPTG, lacI			200
1☆	W	BBa_R0011	Promoter (lacI regulated, lambda pL hybrid)					55
1☆	W	BBa_R0040	TetR repressible promoter	Forward	aTc, tetracyline			54
1☆	W	BBa_R0051	promoter (lambda cI regulated)	Forward	lambda cI			49
1☆	W	BBa_R0053	Promoter (p22 cII regulated)					54
A		BBa_I1010	cI(1) fused to tetR promoter					834
A		BBa_I1051	Lux cassette right promoter					68
1☆		BBa_I12006	Modified lamdba Prm promoter (repressed by 434 cI)					82
1☆		BBa_I12007	Modified lambda Prm promoter (OR-3 obliterated)	Forward	cI			82
1☆		BBa_I12036	Modified lamdba Prm promoter (cooperative repression by 434 cI)					91
1☆		BBa_I12040	Modified lambda P(RM) promoter: -10 region from P(L) and cooperatively repressed by 434 cI					91
A		BBa_I13005	Promoter R0011 w/ YFP (-LVA) TT					921
A		BBa_I13006	Promoter R0040 w/ YFP (-LVA) TT					920
1☆		BBa_I14015	P(Las) TetO					170
1☆		BBa_I14016	P(Las) CIO					168
1☆		BBa_I14017	P(Rhl)					51
1☆		BBa_I14018	P(Bla)					35
1☆		BBa_I14033	P(Cat)					38
1☆		BBa_I14034	P(Kat)					45
A	X	BBa_I714890	OR321 of PR and PRM					121
A		BBa_I714925	RecA_DlexO_DLacO2					862
A		BBa_I714926	RecA_DlexO_DLacO3					862
A		BBa_I714928	RecA_S_WTlexO_DLacO2					862
A		BBa_I714931	RecA_D_consenLexO_lacO2					862
A		BBa_I718018	dapAp promoter					81
A		BBa_I720001	AraBp->rpoN					1632
A		BBa_I720002	glnKp->lacI					1284
A		BBa_I720003	NifHp->cI (lambda)					975
A		BBa_I720005	NifA lacI RFP					3255
A		BBa_I720006	GFP glnG cI					2913
A		BBa_I720007	araBp->rpoN (leucine landing pad)					51
A		BBa_I720008	Ara landing pad (pBBLP 6)					20
A		BBa_I720009	Ara landing pad (pBBLP 7)					23
A		BBa_I720010	Ara landing pad (pBBLP 8)					20
1☆		BBa_I721001	Lead Promoter					94
1☆		BBa_I723020	Pu					320
A		BBa_I728456	MerRT: Mercury-Inducible Promoter+RBS (MerR + part of MerT)					635
1☆	?	BBa_I741018	Right facing promoter (for xylF) controlled by xylR and CRP-cAMP					221
A		BBa_I742124	Reverse complement Lac promoter					203
1☆		BBa_I746104	P2 promoter in agr operon from S. aureus					96
1☆		BBa_I746360	PF promoter from P2 phage					91
1☆		BBa_I746361	PO promoter from P2 phage					92
1☆		BBa_I746362	PP promoter from P2 phage					92

The list will look similar to the one in the screenshot above. Each row will contain the length of a particular DNA part (in **base pairs**), a short description of that part, and its BioBrick number, as well as some additional information. When a row begins with a green box marked "A" you know that part is available from the registry, either in a kit plate or by request. A star means that the part has been rated by other teams and found to be functional and of high quality. A green box with a "W" means that the part has been shown to work, while a red "X" means that the part does not work. A yellow box with "?" means that the part's functionality is questionable. A row starting with a blank box may be bad news. You can still contact the registry about the part; however, don't be surprised if it is unavailable.

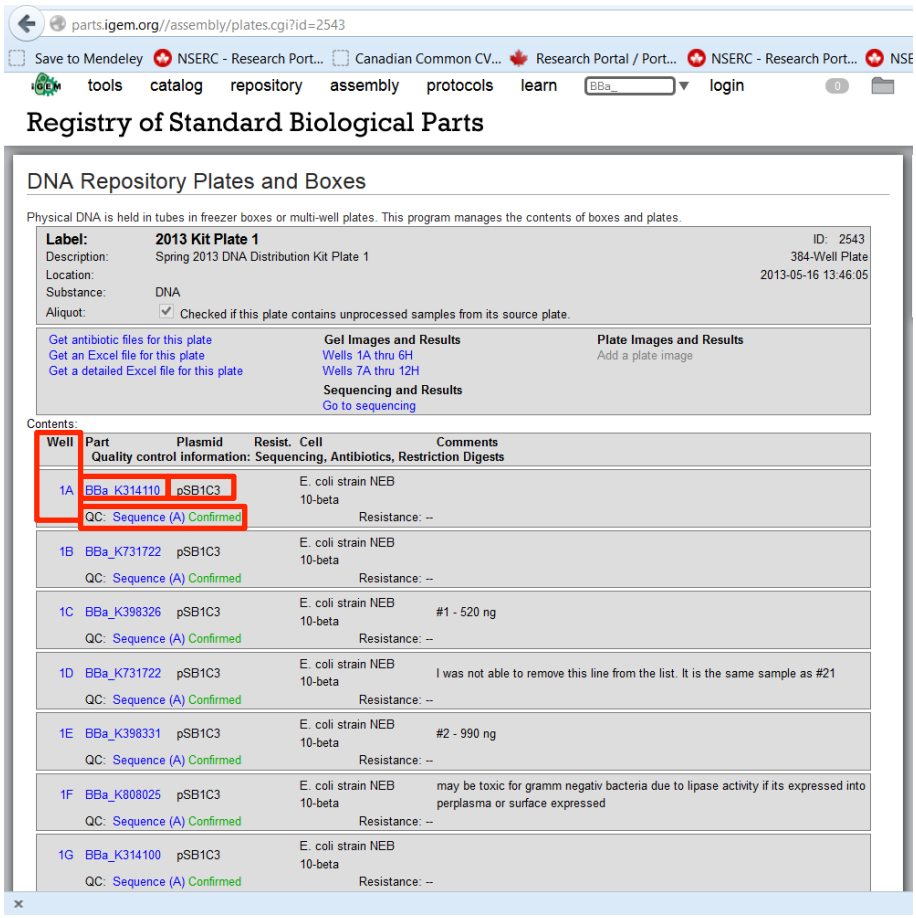
A blank box may also mean that the part was not tested, or the information may not have been updated. In this case you will want to check the parts page for more information. By clicking on the Bba_ links, you will be taken to an information box about that part. By clicking on the part number in this box, you will be taken to the page for that particular part.



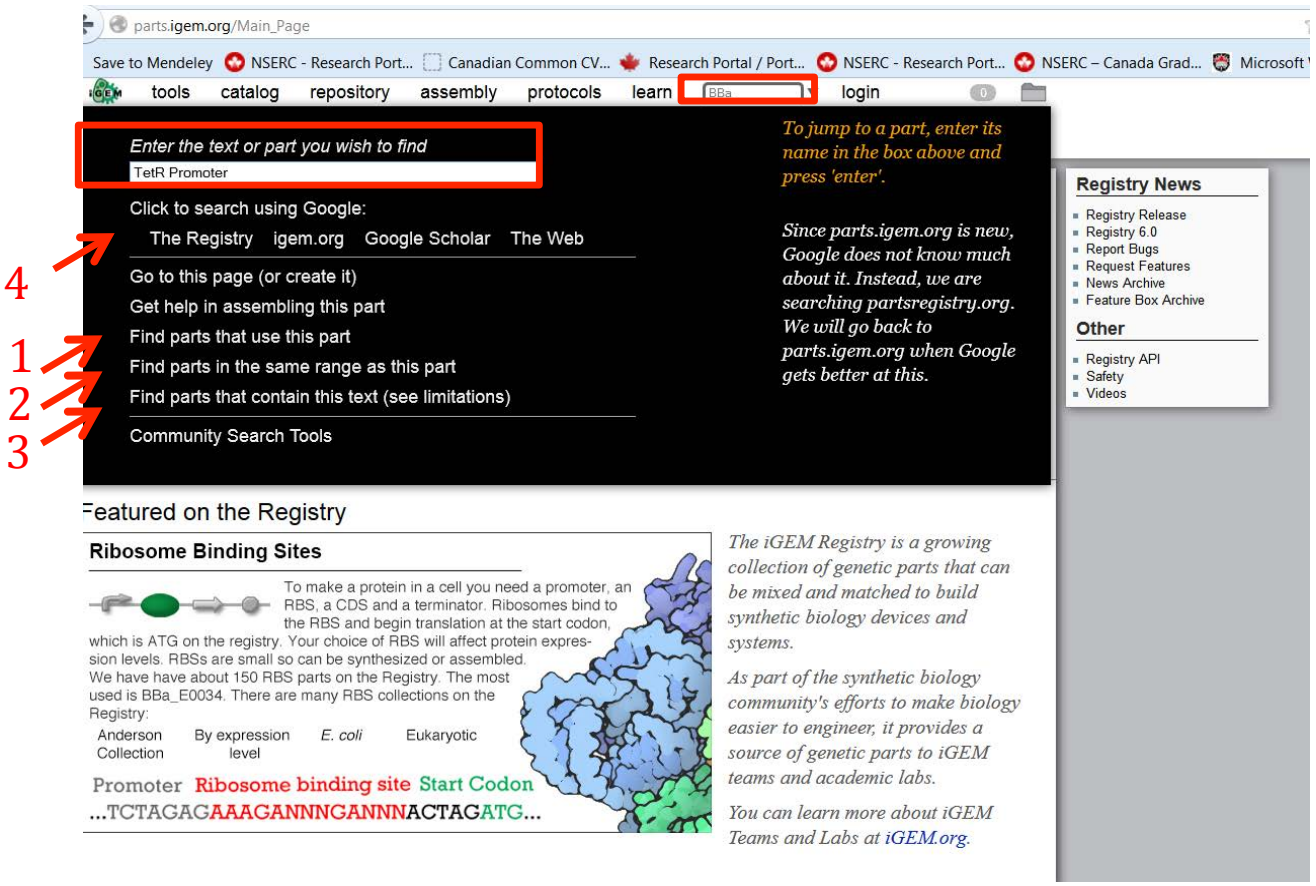
The repository menu on the home page will allow you to search the parts found in the current DNA distribution kit plates, or it will display all the parts in a particular distribution. By clicking “Search Distribution”, you can search for a particular part to see if it was sent in the kit plate. Clicking on a particular distribution will take you to a page with further information.



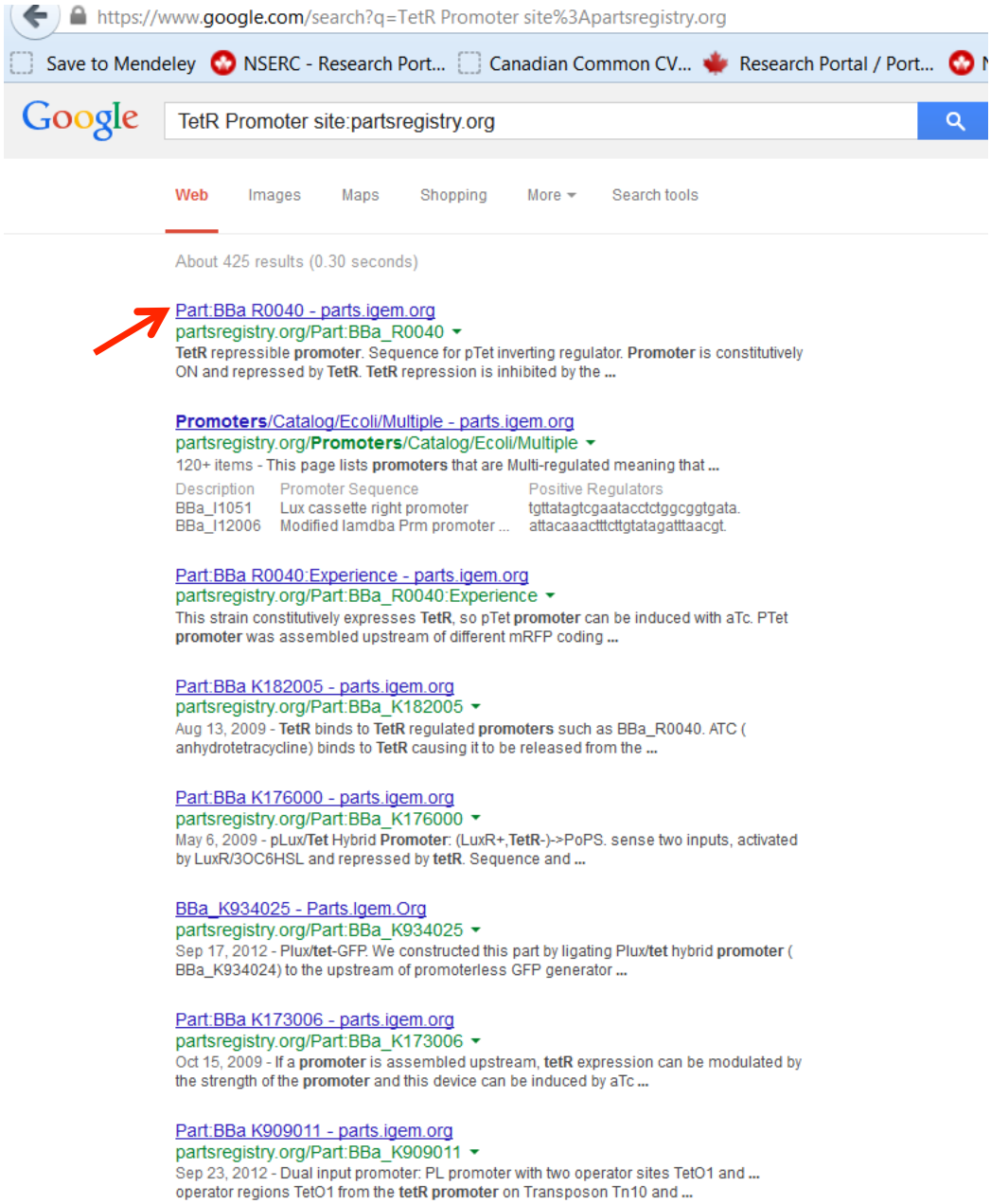
Each distribution is composed of plates, where each well has a single type of plasmid containing a particular DNA part. By clicking the kit plate of interest, you will be taken to a menu that displays what is contained in each well of that particular plate.



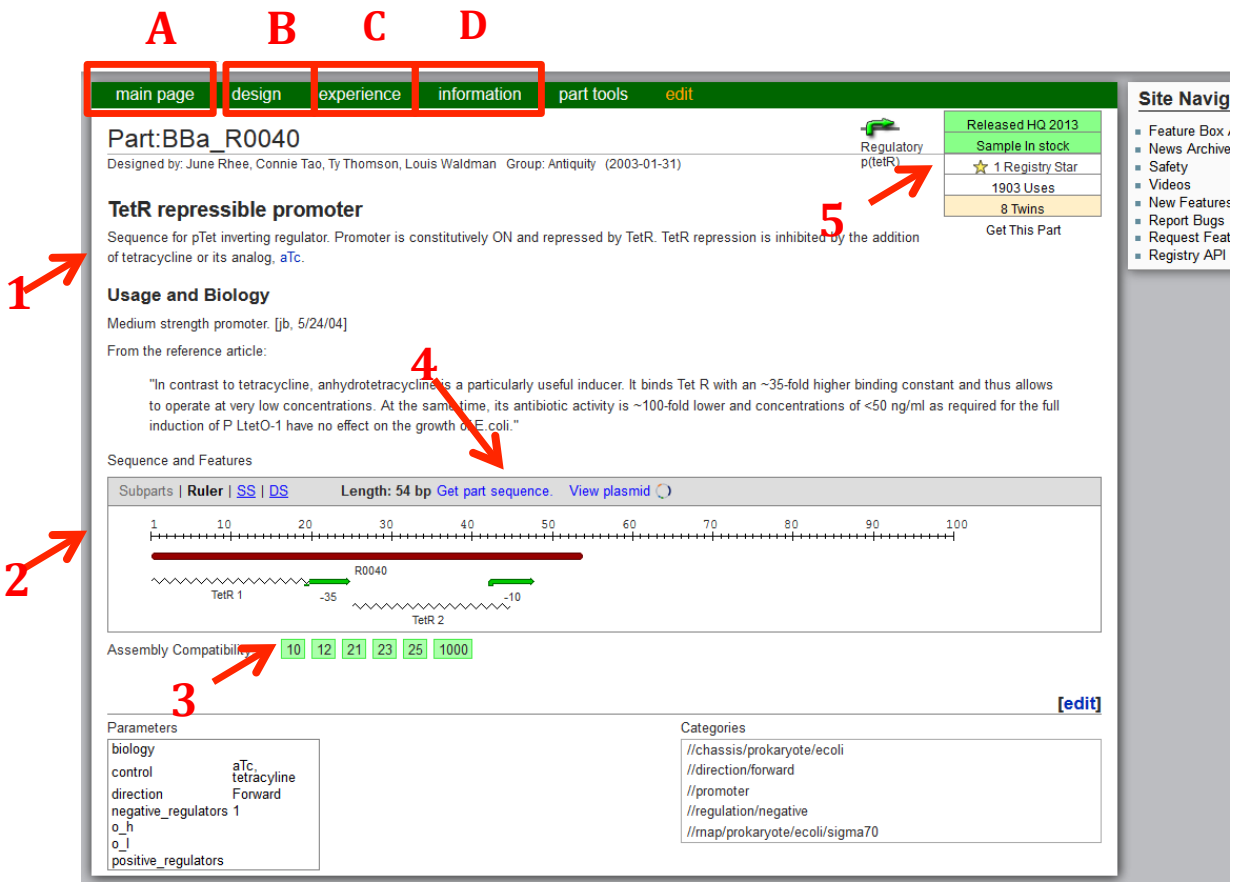
Contents of a kit plate will be displayed by identifying the well in the kit plate in which the DNA part is found, the part's name, information on whether the DNA sequence was confirmed (is the part what it is supposed to be? Note: Longer DNA parts may look like they have bad sequences here even if they are accurate), the plasmid backbone it is in, which microbial strain the plasmid was shown to grow in, and any additional information on how that part works (e.g. is it toxic to the **cells**?). The plasmid backbone name will include a letter indicating the **antibiotic resistance**: commonly it will be resistance to chloramphenicol (C), as PSB1C3 is the standard backbone used; however, occasionally you may find parts in PSB1A3 (ampicillin resistance), PSB1K3 (kanamycin resistance), or in a plasmid with multiple resistances like PSB1AK2 (both ampicillin and kanamycin resistance). Knowing the specific antibiotic-resistance will be important when picking the antibiotic you will need to add to the LB-Agar plates on which you will grow the bacteria transformed with this particular DNA. Clicking on a part name will then take you to the main page for that part.



The search box will allow you to search for parts by number, if you know it. By clicking the arrow next to the part search box, an extended menu will open. In this menu, if you know a particular part name, you can search 1) for larger constructs that contain this particular BioBrick, 2) for parts in the same number range (generally these would have been submitted by the same team that submitted the original part in the same project, and therefore sometimes may involve the part you are searching), or 3) for items containing a certain keyword. If the only information you have about a part is its function, you can search the entire registry by keywords related to that function (4). For example, a search of the entire registry can be done for a tetracycline repressible promoter (this will give you the widest range of results).

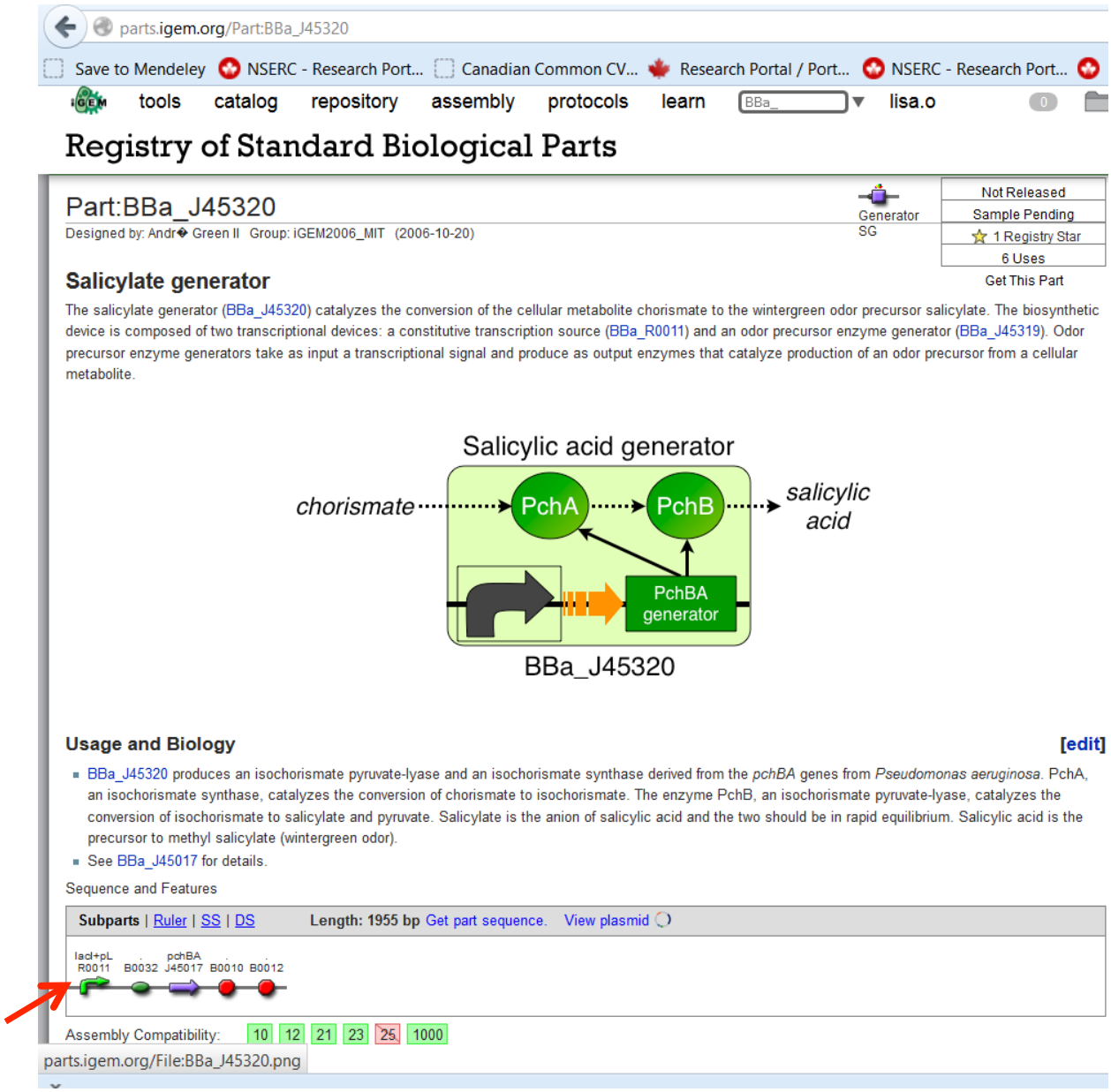


The search engine will give you results that match the text that you have entered. Alternatively, you may search a part directly in Google by typing the same search terms (e.g. “tetracycline repressible promoter”) followed by “parts registry”.

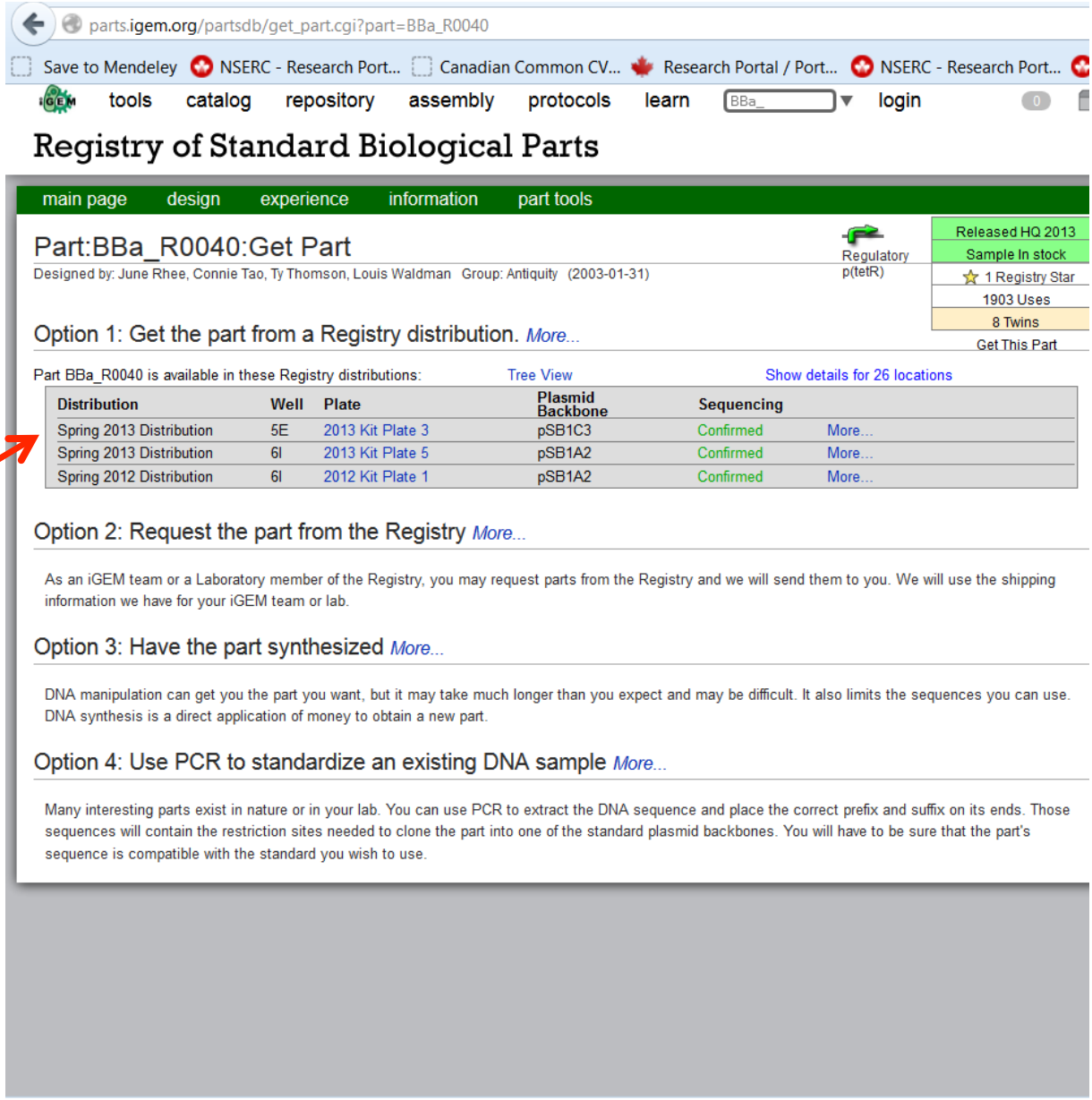


Clicking on this will bring you to the main page for a particular DNA part (A). On this main page, you will find all the information available on that part. You'll find the number of the part, its name, and description of what it is/does (1). You will also find information on the composition of the part, certain features present in the sequence that have been annotated, and the length of the sequence (2). There will also be information about its compatibility with various assembly standards (3). By clicking on "Get part sequence", you will be able to see the DNA sequence of the part starting immediately after the BioBrick prefix, and ending right before the BioBrick suffix (4). Information about the part can be found in the top right corner (5) including whether the part was released in a distribution plate, if it is in stock at the registry, if it has any registry stars associated with it, the number of times it has been "used" (mentioned), if there are any twin parts with the same sequence, and a link for ordering the part. Clicking on this last link will take you to a page, where you can either request the part, or find out where it is located in a kit plate from a distribution.

The "design" page (B) will give you more information about how the part was designed, "experience" (C) will give you information about the function of the part and how other iGEM teams have used it, and the "information" section (D) will provide additional data.



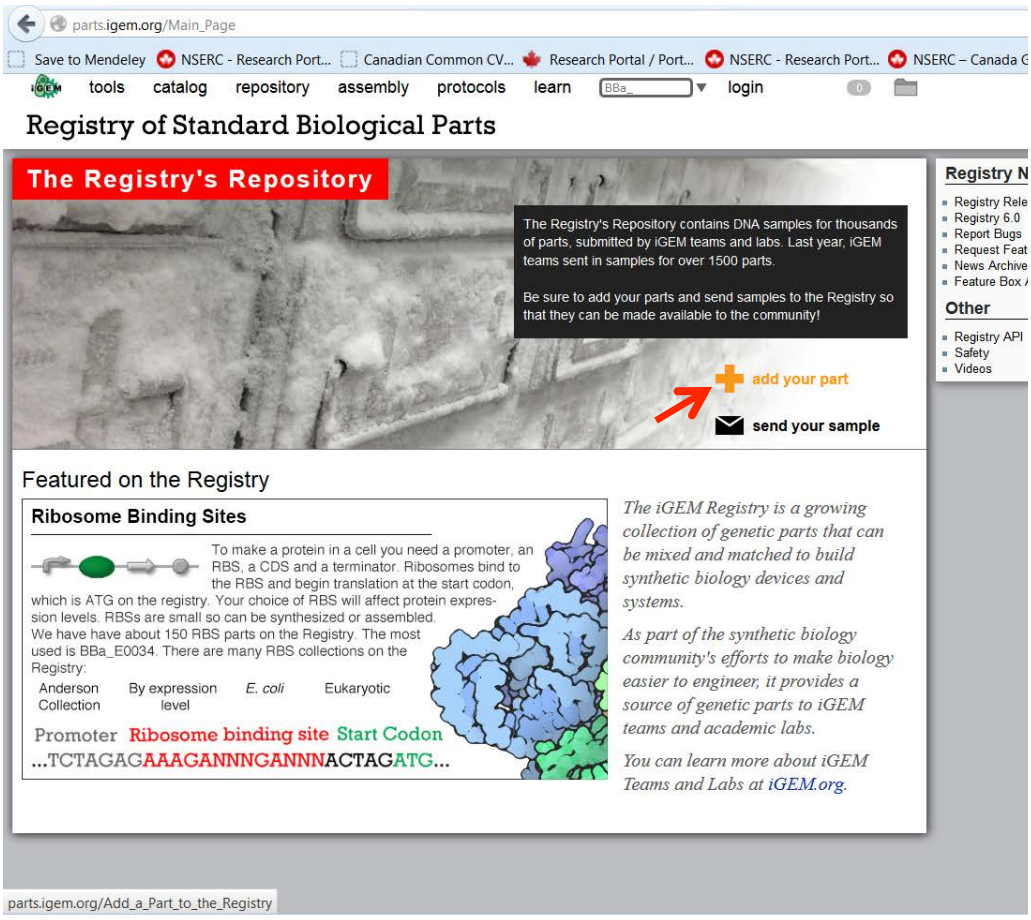
For a composite part made of several BioBricks, the part information display will show you the subparts that it is composed of, and a symbol to indicate what type of part it is. By clicking on any of the subparts you will be redirected to the main part page for that particular subpart.



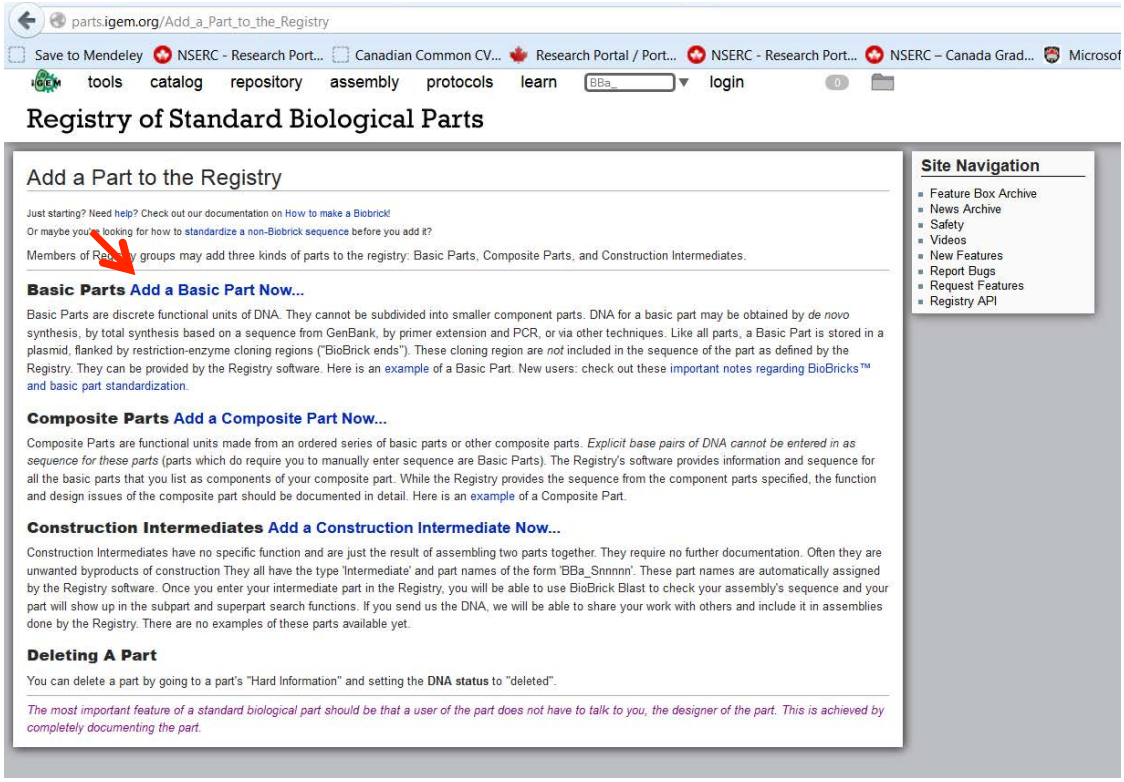
By clicking on the "Get This Part" link, you can find out if the part has been released in a distribution kit, and if yes, which plate and well it is in, what is the plasmid backbone (and therefore, what **antibiotic resistance** gene it carries), and if its sequence has been confirmed. On the other hand, if the part you are looking for is not available in your distribution kit, you can use this page to request it from the registry, as well as find out about other options for obtaining it.

4. How do we submit our own Biobrick parts to the registry?

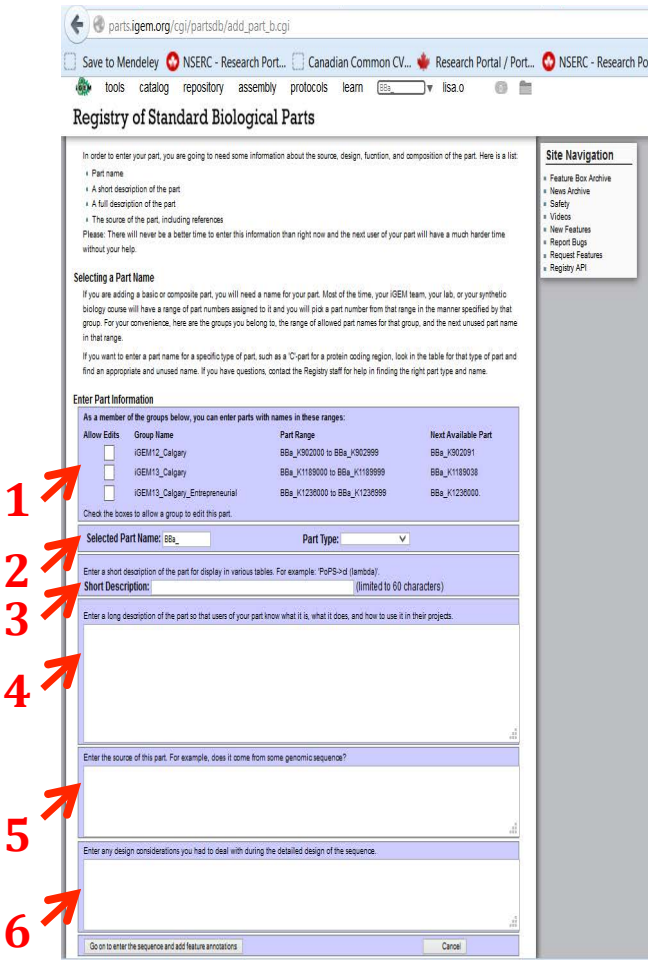
During your project, you will be building one or more BioBricks. You are then required to submit these parts to the registry by a certain deadline, by creating a page describing each part and by sending part DNA samples to the registry.



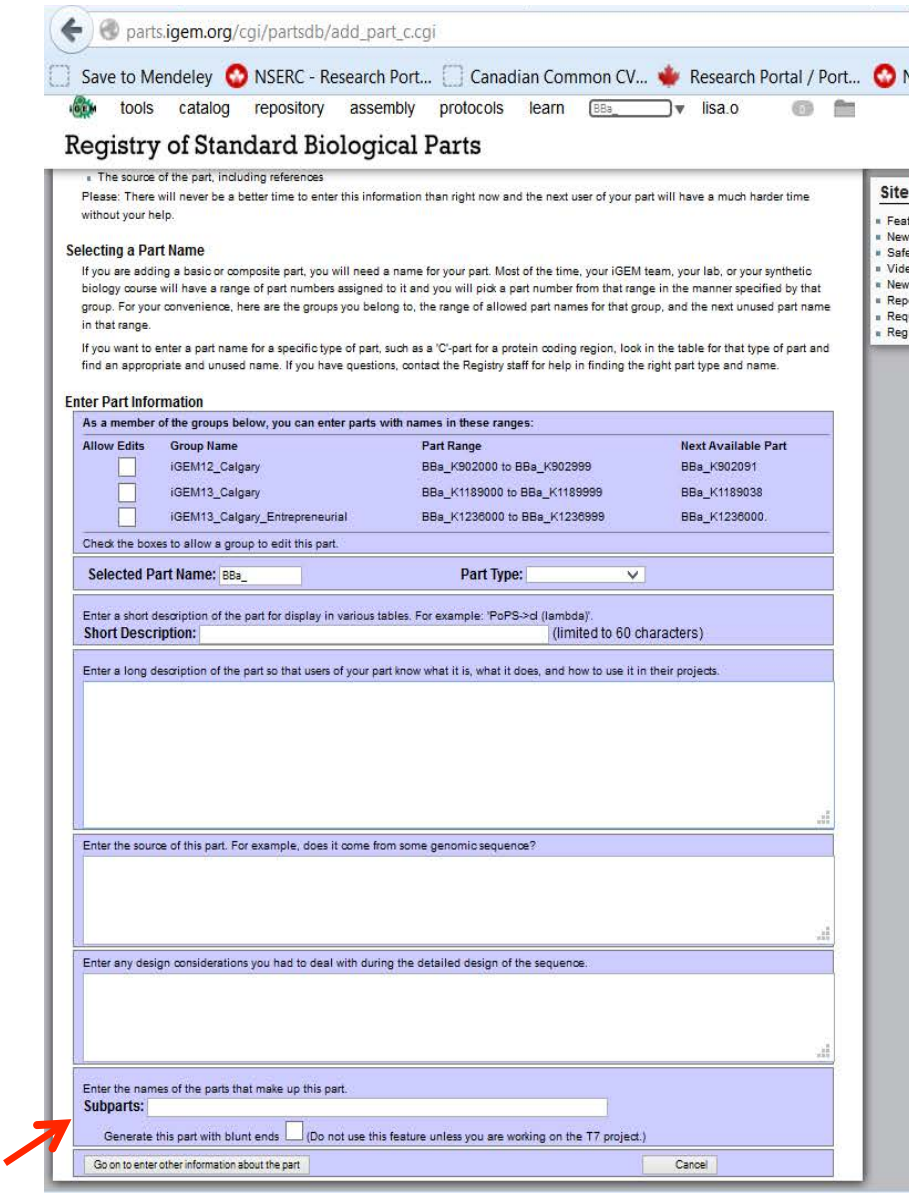
The first step of this process is to create a parts page for your particular part, through the “add your part” tool. In order to do this, make sure you are logged in.



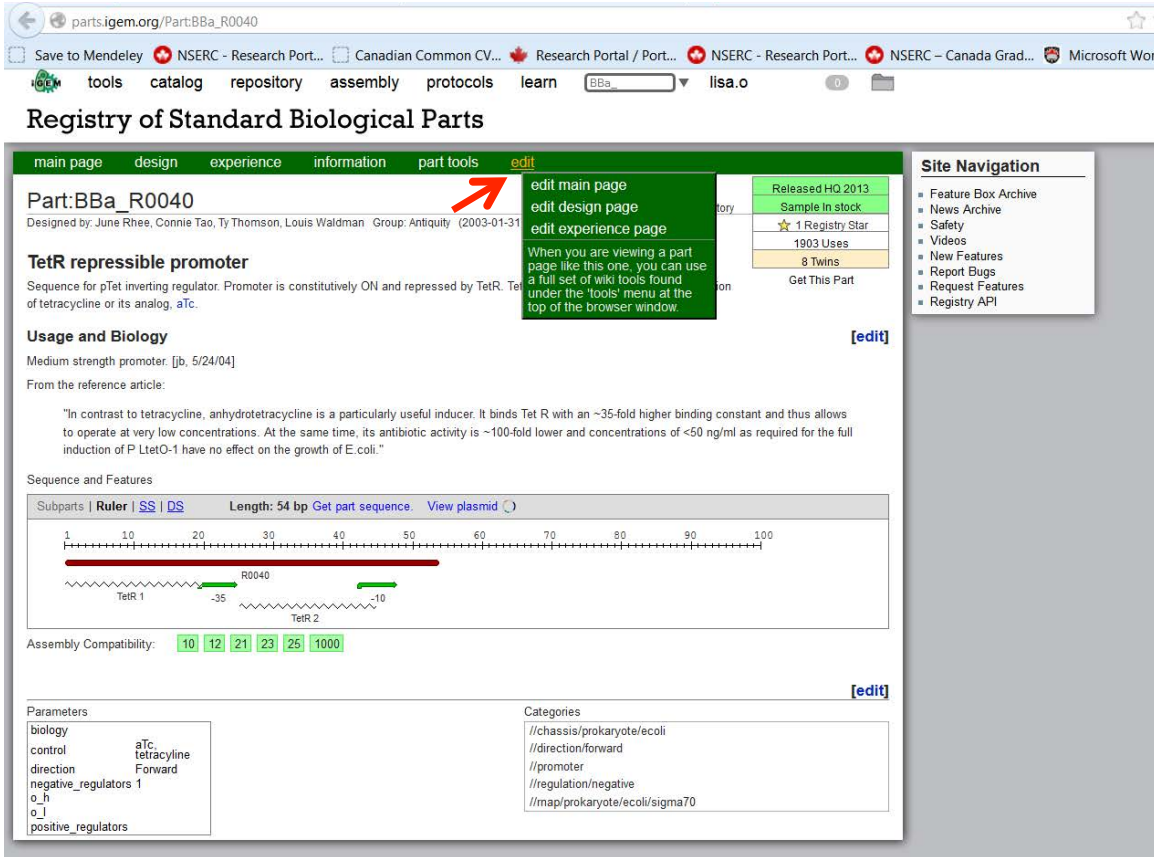
There are a several types of parts that you can submit. A basic part usually consists of a single gene/part. A composite part is a part built out of other BioBricks as subcomponents, and usually contains more than one functional unit. A construction intermediate is a part that you created during the process of building a functional composite part, but that on its own has no specific purpose or function. Generally, the first parts you will need to create are basic parts. You cannot create composite parts without first creating the basic parts that make up the composite parts you are submitting.



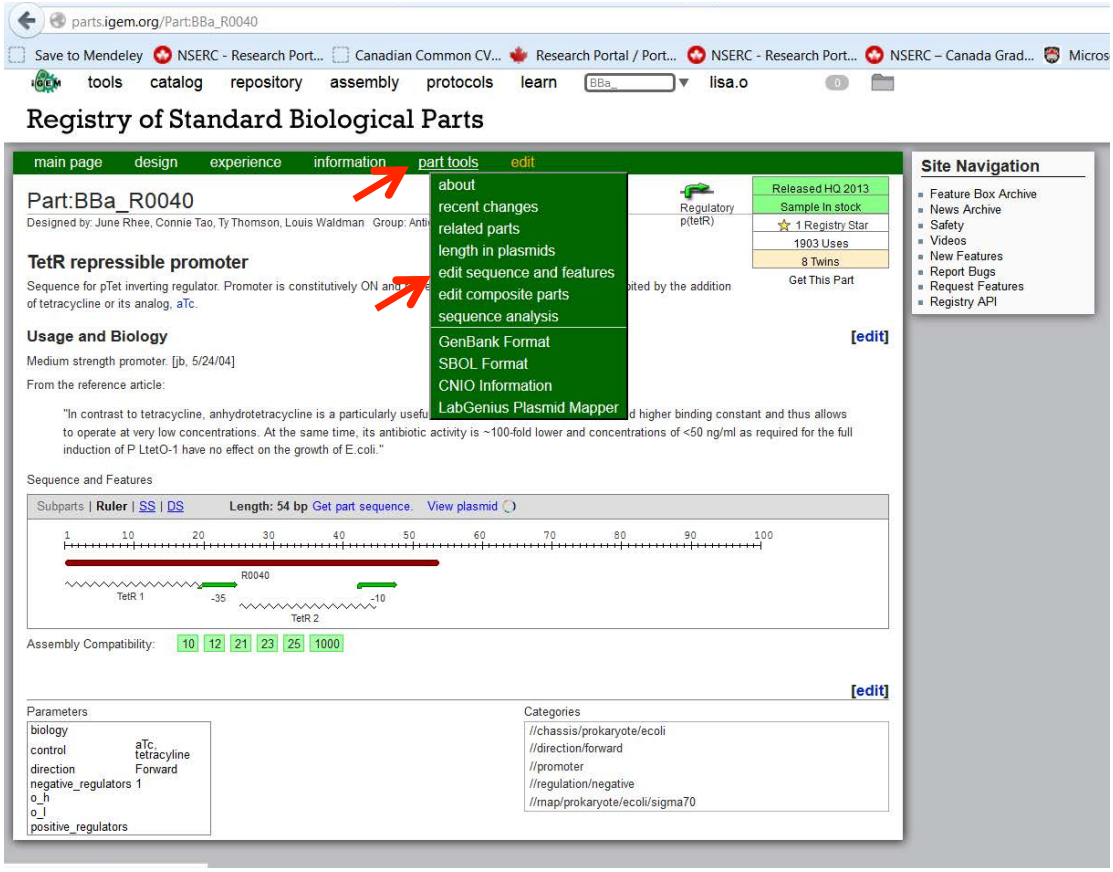
For a basic part, the following areas of information will need to be filled out. The first box that you must check (1) will indicate which team you belong to, as well as the range of part numbers you are designated to use. If your team has created other parts pages already, it will also tell you the next available number you can use. The next area (2) is where you input the number for the part that you are entering, based on the designated range. It is here that you also select what type of part it is - A promoter, coding sequence for a gene, etc. Next (3), you must provide a short description of the part, which will essentially become its name. Try to be descriptive but concise (i.e. don't just put the gene name, but also what that protein is in full). Since you will not be able to easily modify this name again, you must be sure of the name you are entering. The next box (4) will be where you type the description of what the part is and how it works, which will show up on the main page. You will be able to edit this information later, however something must be written in the boxes initially, but must be present. In the next field (5) you must describe the source of the part (e.g. did you isolate it by **PCR** from the **genome** of a particular organism, or did you synthesize it? What organism is it from?). In the final section (6) you will need to enter specific details used in the design of the part (i.e. did you add a purification tag, or a useful restriction site, or did you have to point-mutate an illegal cut site from the sequence and where was the change made?). Clicking "go on to enter specific sequence and annotations" will then bring you to an area where you can enter the DNA sequence for your part (from immediately after the BioBrick prefix [usually the **start codon**] to immediately before the BioBrick suffix). As well, this next section will allow you to annotate features related to specific regions of your sequence (such as a specific active site or binding site). For an example, see the R0040 promoter http://parts.igem.org/Part:BBa_R0040.



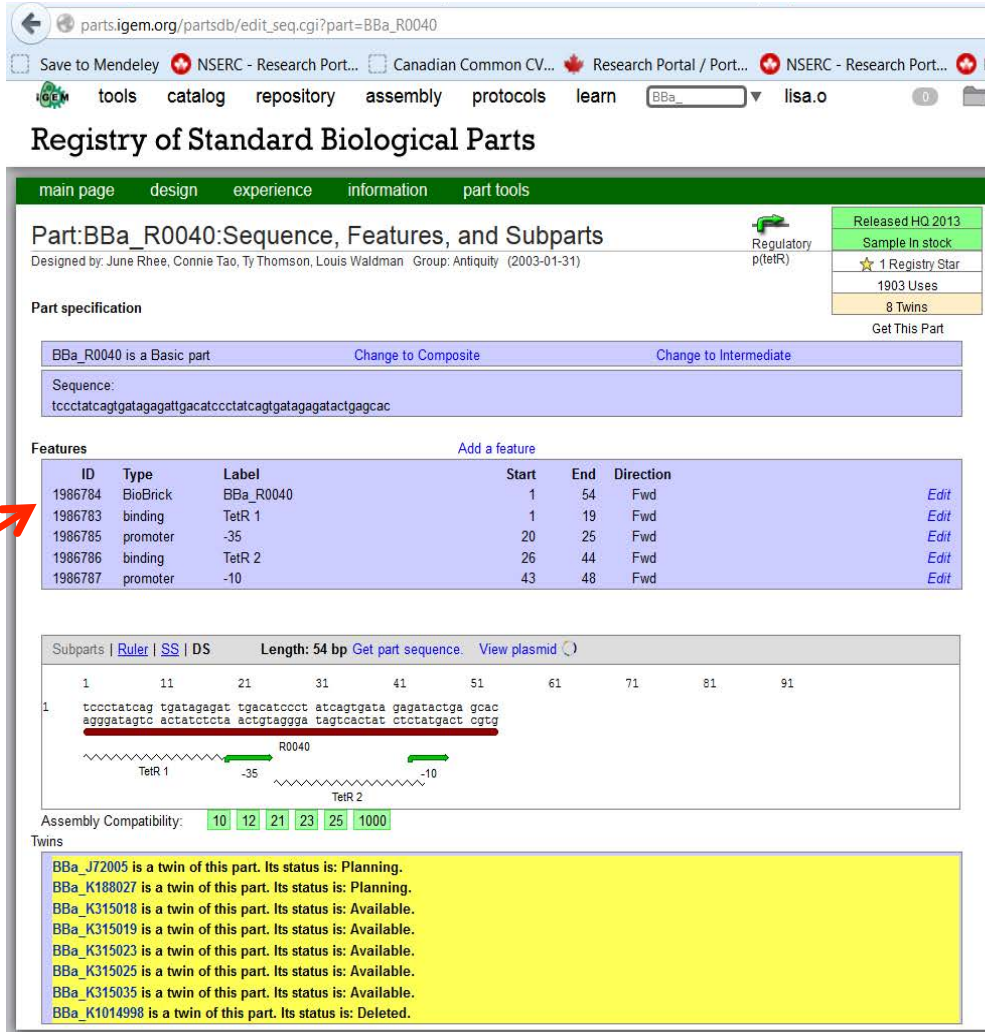
When entering a composite part instead of a basic part, the form is nearly the same, except for an area at the bottom where you will enter the BioBrick numbers for the subparts that the composite is made up of. Enter them in the order in which they are found in the sequence of the composite part. This will also automatically generate a DNA sequence for your composite part from the sequences of the component subparts.



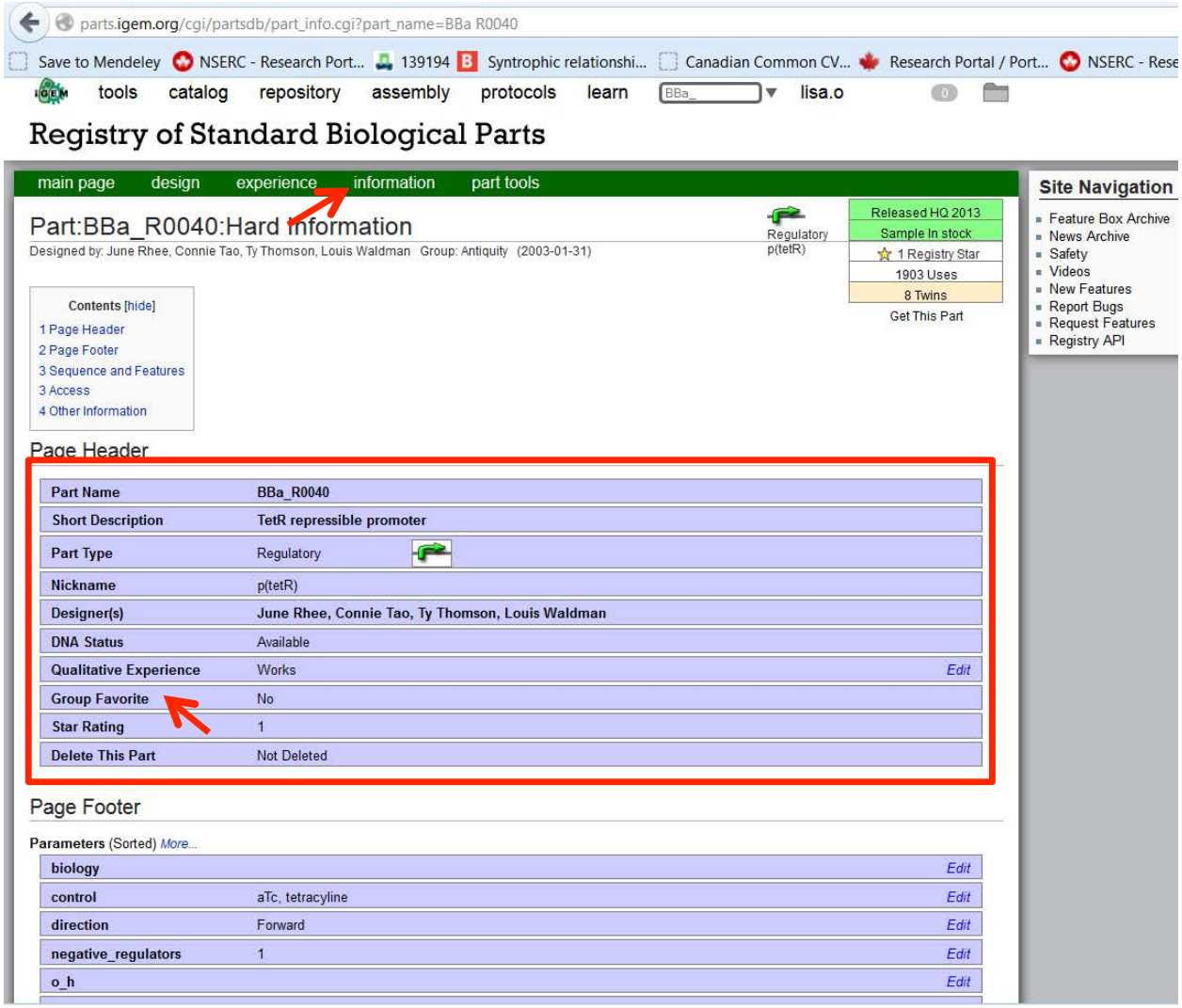
Once you have finished submitting a part page, and entering in all the information, the part will have its own page in the Registry. It is important that you document all of your parts properly. In order to do this once a page has been created, you can use the "edit" menu. The links here will allow you to edit the main, design, and experience pages. The "main" page should have a description of your part, what it does, and how it is used. Also, any characterization data should be entered here as well. Characterization data must also be entered on the "experience" page. The design page will have information about where the part came from, any design considerations, as well as a required reference to where you found the information/idea/sequence of the part, and any citations you use in describing it. Make sure you reference at least one source. These pages are coded in a similar fashion to the **wiki**; however, if you decide to code in html, you must make sure that the <html> tag comes before what you are typing, and the </html> tag comes after.



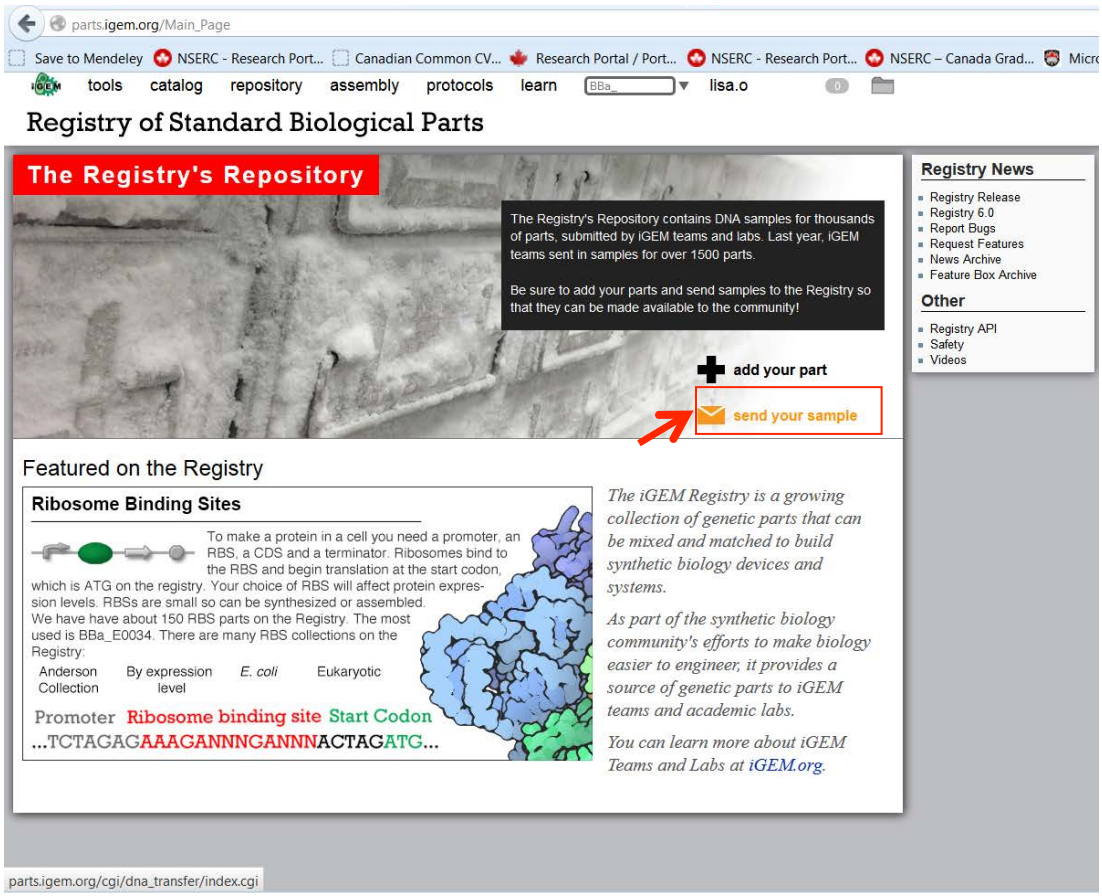
Additional pages that need to be edited are found under the "part tools" menu. The "edit sequence and features" area will allow you to edit the DNA sequence you entered, as well as allow you to annotate any important features of that sequence.



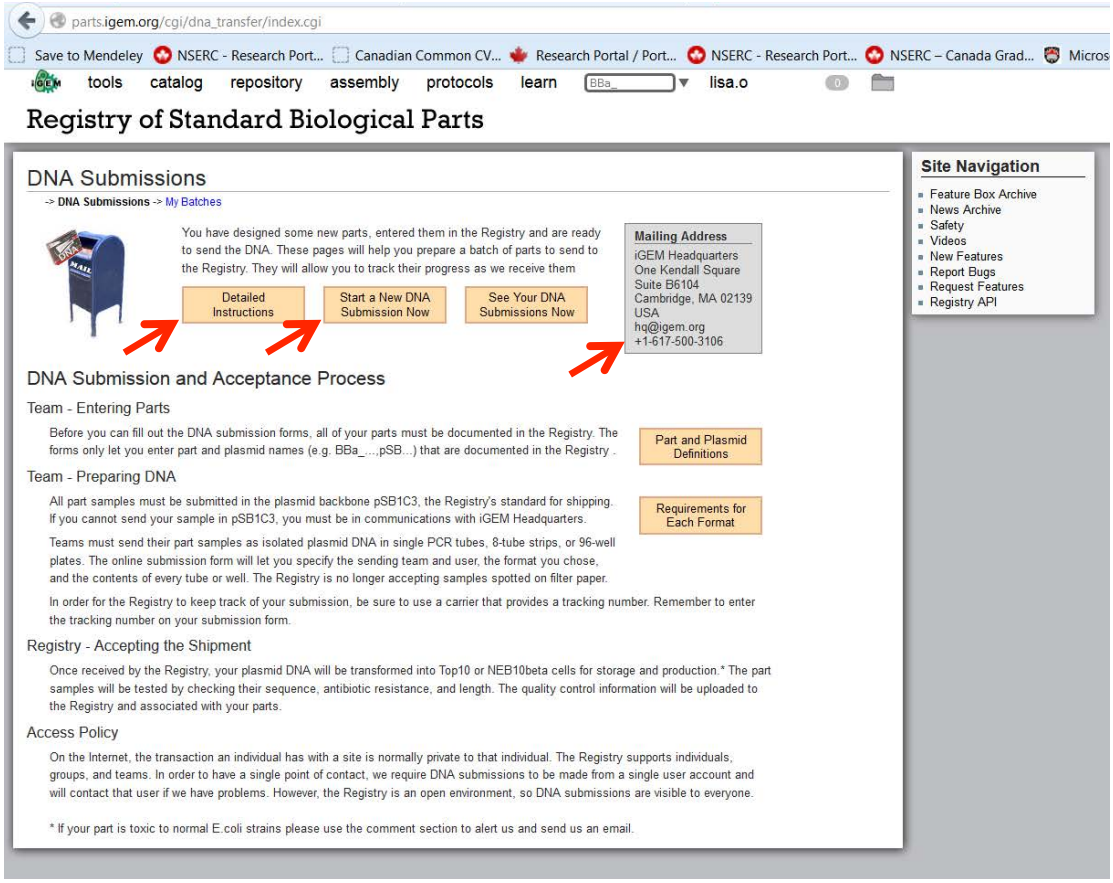
In order to add a feature, you must select what type of feature it is, name it, as well as put in the **nucleotide** numbers of where it begins and ends at along the full sequence.



The information page will allow you to edit the hard description of the part, such as its name, the type of part, who on your team created it, and whether the part works or not. This is also where you can delete a part if necessary. On this page, you can also rate a part as a group favorite - this should be done for the parts that worked the best and provided the most valuable data for your project, as this will flag them for close inspection by the judges.



After you have created part pages for all the parts you are submitting, you can move on to actually sending those samples to the registry. Be mindful of the deadline and the time zone - the parts must be AT THE REGISTRY by that date and time! Individual part pages must have been created for all the parts you want to submit, and all sequences must be entered before you can fill out the form. However, you should know that the information you put in can be expanded on at a later date but should be completed as thoroughly as possible before Wiki-Freeze, as that is when judging begins. Note: All parts submitted to the registry MUST be in the PSB1C3 backbone.



On this page, you will find all the information you need to successfully submit a DNA part. The "Detailed Instructions" section will walk you through the submission processsend. "Starting a new submission" will take you to the form that you need to fill out. The mailing address of iGEM HQ is found on the right. Before submitting parts, talk to your post office or mailroom to make sure you get all the required forms needed for sending DNA samples. Mention that it is DNA you are mailing to get the proper forms, and make sure that it will make the deadline. On all forms that ask what you are shipping, make sure to write "Plasmid DNA, non-toxic, non hazardous, for research purposes only". Don't ever write the words E.coli, or bacteria, as this will result in customs holds and charges! You will also need to assign some kind of value to your DNA samples - generally $1.00/tube or less.

5. A Word on Quality

While acknowledging that the registry is a fantastic toolkit for iGEM teams, it should be noted that, despite efforts to ensure it, quality cannot be guaranteed. It is always advisable to sequence parts obtained from the registry, especially if they have not been used by more than one team. In addition, it is a good idea to try to select commonly used parts whenever possible. As you do your searches, you will learn that many DNA parts have 'twins', meaning that the same part has been submitted by multiple teams. If that is the case with a part that you wish to use in your project, try to pick the twin that has the most data available (look for data on the experience page for the part), or use the one that is marked with a green 'W' (for working). This will increase the chances that it is a good quality part. When a part doesn't have a twin, be cautious about using it if there is little or no data for it, or isn't marked with 'W'. This could mean that they are not good quality, which may impede your progress. Finally, you will find that for some parts there isn't much information available. Try to avoid using those as much as you can, and use parts that do have the information you're looking for. And to save yours and other iGEMers time in the future, when you create and submit your own DNA parts, ensure that what you enter has a ton of meaningful, carefully written information, and that the

DNA sequence is accurate and entered correctly. This will help other teams in the future and it will increase the overall quality of the Registry, which will be essential for the progress of synthetic biology.

7. BIOSAFETY

David Lloyd

Why **Biosafety**?

Working in a lab comes with its own unique set of experiences, some of which can be harmful if you do not know what you are doing! This section of the guidebook will focus on safe practices for performing molecular biology within your high school space.

Please note: If you are ever unsure of anything iGEM-related that you are doing in your lab space, talk to your mentors! This is incredibly important to keep you safe from your bacteria and to keep your bacteria safe from you (we don't want to get our cultures contaminated)!

This section will also provide a more broad definition of biosafety in the context of the competition, and will describe what the judges will be looking for when evaluating this aspect of the **Human Practices** component of your project.

Step 1: Review the iGEM Biosafety Information Sheets

If you have not already done it, be sure to check out the biosafety resources available to you as part of the iGEM 2014 information page which can be found here: http://2014hs.igem.org/Safety

Also it is a good idea to check out the parts registry information pertaining to biosafety and **biosecurity** here: http://parts.igem.org/Biosafety

Step 2: Introducing Biosafety into Your iGEM Project

Hopefully these pages will convince you of how important biosafety is and why it must play a prominent role in your project. While brainstorming project ideas, be sure to ask questions on how your ideas might affect the world:

1. Who or what will be affected by the organism we are planning to create? (Environment, populations, animals, etc.)
2. How will this organism affect other organisms and the environment?
3. What are the potential negative consequences of what we plan to do, no matter how rare or unlikely?
4. What can we do to minimize the risk of such negative consequences?
5. How will our organism be viewed by the public and people in our community?
6. Which would be an ethical way that we can use our organism to better our world?

It will be of essence to consider such questions early on when designing your system to ensure that your final organism has a good chance to be adopted by society and that it will not be perceived negatively due to potential risks.

This incredibly important element of the competition will be judged in your **human practices** section. The Human practices section includes the social, legal, ethical, and safety elements of your project. You should not be surprised to learn that judges take this component of projects very seriously as it is critical that individuals who work in **synthetic biology** consider the far-reaching implications of their work beyond the laboratory.

People tend to misunderstand human practices and think of it as a community **outreach** component. As a result, many iGEM teams have gone to high schools, middle schools, or different community groups to teach about the new field of synthetic biology. However, the human practices component is about much more than simply sharing knowledge about synthetic biology. Judges will be looking for how your human practices elements have benefitted your project, how they have influenced decisions you have made in regards to building your organism, and how your project specifically fits into the world around us. Therefore, it is highly recommended that you start with a comprehensive review of biosafety as it applies to your lab, not only in regards to the new organism you are planning to make but also to the methods and procedures that you will need to use.

You will be required to submit safety information to participate in the competition for which it is recommended that you consider the above.

Step 3: Designing A Safe Space To Work In

Your lab space must be adequate for the molecular biology work that will be going on.

If your school or organization has a safety committee, please ensure that you have communicated with them about the types of experiments and all the lab work that will be done in the classroom. Ensure that you meet all the safety standards established by your safety committee.

If your safety committee can provide documentation about your lab space, be sure to make this publicly available on your wiki.

Basic Requirements of a space:

1. All surfaces should be clean and disinfected, and it is recommended that you work on a surface that is non-absorbent and non-flammable. Coat the surface in bench coat paper to make cleaning easier and to contain any contamination.
2. Store chemicals away from any sources of gas or flame. We will touch more on proper handling of chemicals later in this section.
3. Ensure that all students wear personal protective equipment appropriate for the activities they will be performing and that they follow personal hygiene rules while in the lab. Gloves, lab coats, and eye protection should always be worn when doing any kind of work in the lab. Long hair should be tied back, glasses should be worn rather than contacts, and no food or drink should be allowed in the lab area.
4. Bags, books, etc. should be in a "work free zone" away from any potential source of contamination.
5. Students should be supervised at all times.
6. Mouth **pipetting** should NEVER be allowed.
7. Emergency procedures in case of fire, gas leak, chemical spill, or injury should be posted in a visible place and easily accessible to the students.
8. Ensure a fully stocked first aid kit is available in the lab and everybody is aware of its location.
9. The lab should be equipped with an eye wash station and shower station to assist in a chemical spill.
10. A spill kit should be available in case a chemical gets spilled onto the floor. This kit should contain:
    a. Paper towel
    b. Kitty litter (or some kind of other material to absorb the spill)
    c. Gloves
    d. Waste bags
    e. Lab coats

f. Goggles
g. A sealable container
h. Sodium Bicarbonate (for acid spill)
i. Citric Acid (for base spill)
j. Dust pan
k. Brush

11. All students should be knowledgeable about the **WHMIS** symbols that chemicals are labeled with.
12. A Biosafety manual should be developed; an example can be found here: https://www.ucalgary.ca/safety/biosafety_manual. This should contain a quick reference of all guidelines and rules in your lab space that is accessible by everyone
13. Only allow trained individuals into your lab space, i.e. only students that have received safety training should be allowed to work with the chemicals, bacteria, or other components.

Chemical Safety:

Chemicals vary with respect to their potential toxicity, and therefore every chemical that you order must be handled with extreme care. Prior to handling a chemical, make sure to read its Material Safety Data Sheet (**MSDS**) to learn how to handle it safely

Keep all MSDS sheets for reference and put them into a place that will be easily accessible in case of emergency. Again all MSDS sheets should be reviewed by all members of the workspace prior to their use.

Below is a list of chemicals you will use, which require particular attention:

1. Sodium Dodecyl Sulfate (SDS) – Very strong detergent. If breathed in a powdered form may cause lung damage.
2. SybrGreen/SybrSafe – Used to illuminate **DNA**. It is generally regarded as safe, but because it can intercalate in the DNA helix and distort it, no direct contact is recommended.
3. HCl – Strong acid which could burn skin if spilled, do not breathe in fumes
4. NaOH – Strong base which could burn skin if spilled

In addition to the more hazardous chemicals listed above, you will be using many others, which should be handled according to the safety rules as well.

Storing of chemicals requires special attention too as some chemicals may be prone to oxidation or they may be strongly reactive when mixed with other chemicals. Always follow the MSDS guidelines for where chemicals should be stored. For example, solvents like chloroform or hexane should be stored within a ventilated fumehood.

Some individuals may have specific allergies to particular chemical components. A common example of this is antibiotic allergy to one of the chemicals you will be using in iGEM. Be sure to inform your instructor if you are potentially allergic to any of these compounds (common examples are ampicillin, or any other penicillin derivative, kanamycin, tetracycline, or chloramphenicol).

Step 4: Aseptic Technique and Sterilization

When it comes to biosafety, things work as a two way street. You do not want to become contaminated or exposed to anything in the lab, but you also need to ensure that you yourself do not contaminate the lab and especially your bacterial cultures. For this you need to use the **aseptic technique** which keeps both you and your experiments sterile.

Here are some examples of how to maintain good aseptic technique:

1. Any object that may or will come in contact with bacteria needs to be sterile. The following can be used to ensure your work is sterile:
    a. 70% Ethanol – A great decontaminating agent that will kill most bacteria, fungi, and other organisms which could contaminate your cultures
    b. Detergents/Cleaning Agents – Good for cleaning lab surfaces
    c. High Temperature and Pressure – Heating solutions to boiling or using an autoclave are great ways of sterilizing materials. Most molecular biology solutions will need to be autoclaved to ensure sterility
    d. Bleach – A bleach solution of approximately 5% is very efficient at killing most living material. Take the necessary precautions when using bleach because it is corrosive!.
    e. Gloves/lab coat/eye protection – Make sure to always follow standard safety protocols to protect yourself. On the other side, it is important that bacteria naturally found on your skin do not contaminate your cultures.
2. When working with live microorganisms make sure you apply the following basic aseptic techniques :
    a. To maintain an area clean and sterile, wipe down surfaces with ethanol or with a detergent product (Lysol, for example).
    b. Only use clean, sterile glassware. This includes any pipette tips, glass and plastic tubes, solutions, and anything else that will come in contact with your bacteria.
    c. Whenever you open up a container you are exposing its inside to the air, which is **non-sterile** (there may be airborne particulates or bacteria that could contaminate your solutions). To prevent contamination, pass the opening of the container through the flame of a Bunsen burner. This will kill off any microorganisms, which could otherwise contaminate your solutions when pouring from one bottle to another.

Step 5: Disposing of Bacterially Contaminated Solutions and Materials

All solutions or materials that have come into contact with bacteria must be separated from other waste materials. All bacteria must be killed prior to disposal and this can be done by either of two methods. The materials can be either autoclaved, or disinfected using a 5% bleach solution. Usually liquids are soaked in bleach for an extended period of time, whereas dry materials are autoclaved. Once disinfected, all materials can be disposed of as regular waste. Please note: If any chemicals are present that should be disposed of separately, the disinfected waste material must be managed according to the type of chemical waste involved.

Always consult a researcher, mentor, and the applicable guidelines when dealing with biosafety issues. If at any point you have questions or concerns about the safety or proper handling of a piece of equipment, chemical, or protocol, do not proceed without the necessary assistance and clarification.

8. TEACHER ADMINISTRATION AND FUNDRAISING

Robert Mayall and Iain George

1. Permissions:

Building a Team

Whether you are building a brand new team, or continuing from a previous year, there are many administrative pieces that your team will need to think about and work through. Many of these pieces traditionally fall towards senior members of the team, but many of the most successful teams share these responsibilities between both teachers and senior students.

Starting an iGEM team has many requirements, both at the level of the competition organizing body (iGEM) and at the level of your institution. The first and foremost objective is to engage the administration within your school, as without their support it will prove challenging to start a team. A good first step would be to discuss the idea with the principal of your school to ensure that entering into a competition such as this is within the purview of your school. An additional follow up step that may be required would be to approach the school board for your district to seek their approval. By engaging with your administration early in the development of an iGEM team you will avoid unexpected obstacles further into the competition season. This may also lead to additional contacts and support networks to help build and fund your team.

Other key stakeholders that should be brought into discussion could include other teachers at your school, alumni from your school that might be interested in supporting the team, and parents of students who have expressed interest in being involved in a team.

The iGEM Foundation, iGEM's organizing body, has many requirements they impose for high school teams that wish to compete each year. A full list of these requirements can be found here: http://2013hs.igem.org/Requirements. These requirements may change over the years, so it would be prudent to look at the requirements for the year of the competition you are seeking to enter. In brief, the requirements are as follows:

1) The team must be made of high school students with a teacher acting as a supervisor.
2) The team can consist of students from more than one high school (if desired).
3) Consent forms must be completed (the following section describes this).
4) The team must register with iGEM and pay a registration fee.
5) The teams are encouraged to attend the high school iGEM jamboree.
6) The project must be documented on a **wiki**.
7) All work must be attributed to the person who completed it.
8) Teams must fill out a safety form (detailed below).
9) The project must be presented (oral and poster) at the jamboree.
10) The team members should enjoy themselves.

Completing an iGEM project will require a significant investment of time from both teacher and students. While the students should be primarily responsible for the laboratory work, they will also

need to create the poster, presentation, and wiki for the team. Regular team meetings, usually once a week, will help to keep the project on track. The time needed per week can vary greatly depending upon the progress of the project and the number of team members, but it is not unusual for teams to spend some long nights and weekends on the project closer to the competition deadline.

2. Forms

There are many forms that have to be completed for the iGEM competition. There are four documents at this link:

1. Competition Information Sheet, which describes the competition and can be handed out to students and parents who are interested in learning about iGEM.
2. Participant Consent Form, which testifies to the student's and instructor's desire to be involved in the iGEM competition. This form also outlines information that may be of particular interest to parents.
3. Principal Consent Form, which addresses liability at the competition.
4. Department Head Consent Form, which is only applicable if the work is being undertaken at a postsecondary institution. This form acknowledges that the head of the department that runs the laboratory being used understands that he/she is responsible for the safety of the students using the laboratory.

The official forms for the 2013 competition can be found here:

http://2013hs.igem.org/Consent_Forms.

An additional mandatory form for all participating iGEM teams is the "Safety Page." The information for this form can be found here: http://2013hs.igem.org/Safety. This form primarily addresses the safety of your project and how you will be ensuring it. Examples of potential hazards that must be included would be the use of hazardous organisms or potentially harmful genes/proteins. The judges at the iGEM competition are more than willing to provide advice with this form, as they wish to ensure that teams compete in a safe way.

Beyond the forms required for iGEM, your school and/or district might impose additional requirements. The best way to determine this would be through discussions with your schools principal or other teachers. These additional requirements could include out-of-country travel forms, biological organism usage forms, or forms required for the purchase of certain equipment or reagents necessary for your project.

3. Lab Safety & Supervision

Before working in any lab, students need to receive the proper safety training. The requirements for each lab will vary, so the administrator for the space you are using will need to be contacted. Almost every lab will require students to receive the Workplace Hazardous Materials Information System (**WHMIS**) training at the bare minimum. After the WHMIS training, courses in **biosafety** may be needed. The level of biosafety training required will depend on the lab and which organisms your project will be using, with more training required for bacteria with higher risk levels. For reference, the majority of iGEM projects are done in "Level 1" or the lowest biological risk category labs.

In addition to the mandatory training, the students will require constant supervision in the laboratory. This is primarily for safety, but also for insurance and liability purposes in most laboratories. The supervisor does not necessarily have to be the primary teacher/advisor though, as any professor, graduate student, or senior undergraduate student associated with your team may

also be appropriate for this purpose. It is highly recommended to discuss supervision with the administrator of the laboratory space you will be using before setting up a supervision schedule.

4. Finances:

Budgeting

An essential part of a project revolves around the management of the funds needed to operate the laboratory. Without money it may prove difficult to obtain reagents, pay the necessary fees, or attend the competition. Key to making the money stretch as far as possible is to keep track of it in a detailed budget. This budget should include every expense that can be planned for, erring on the side of caution with price estimates. An example budget for an iGEM team is shown below. This excel file can be made available upon request as a template for your team.

Example Budget Sheet - HS iGEM 2013						
		Secured				
	Cost	VWR	AITF	Funding required	Genome Alberta Request	Deficit
Travel to iGEM Jamboree (Cambridge, MA, Nov 1-4, 2013)						
Airfare (10 students + 2 TAs + 2 Instructors, 14 x $800)	11,200.00		5,000.00			
Travel insurance for students (12 x $25)	300.00					
Ground transportation (4 taxis x $40 x 2)	320.00					
Meals	300.00					
Hotel rooms (5 rooms x 4 nights x $179 x 20% estimated tax)	4,296.00					
iGEM conference fees (14 x $90)	1,260.00					
	17,676.00	0.00	5,000.00	12,676.00	**10,000.00**	2,676.00
Travel to Edmonton workshop (Edmonton, AB, May 11-12, 2013), AITF Mandated						
Hotel rooms (7 rooms x 2 nights x $65 x 5% tax)	955.50		955.50			
Meals	150.00		150.00			
Gas (4 cars x $0.46/km x 220km x 2)	809.60		809.60			
	1,915.10	0.00	1,915.10	0.00	**0.00**	0.00
Travel to Lethbridge workshop (Lethbridge, AB, July 20-21, 2013), AITF Mandated						
Hotel rooms (6 rooms x 2 nights x $140 x 5% tax)	1,764.00		1,764.00			
Meals	150.00		150.00			
Gas (5 cars x $0.46/km x 300km x 2)	1,380.00		1,380.00			
	3,294.00	0.00	3,294.00	0.00	**0.00**	0.00
Travel to aGEM (Edmonton, AB, Sept 14-15 (*tentative*), 2013), AITF Mandated						
Hotel rooms (6 rooms x 2 nights x $140 x 5% tax)	1,764.00		1,764.00			
Meals	150.00		150.00			
Gas (5 cars x $0.46/km x 220km x 2)	1,012.00		1,012.00			
	2,926.00	0.00	2,926.00	0.00	**0.00**	0.00
Supplies and Materials						
kits and reagents for TALE screening	1,000.00	1,000.00				
Kits and reagents for protein purification	750.00	750.00				
Primers	500.00					
Sequencing service	750.00					
Molecular biology supplies	750.00	750.00				
Consumables	750.00					
Prototype development	250.00					
T-shirts	250.00					
Poster printing	100.00					
	5,100.00	2,500.00	0.00	2,600.00	**2,600.00**	0.00
Other						
Team registration fee	1,500.00		1,500.00			
	1,500.00	0.00	1,500.00	0.00	**0.00**	0.00
Totals:	32,411.10	2,500.00	14,635.10	15,276.00	12,600.00	2,676.00

Fundraising

Fundraising can seem like an enormous task in the beginning, but fundraising for your team is one of the most important components of a successful run. As you will learn here, having enough funds is essential to the many steps along your way, from purchasing supplies and equipment to covering travel expenses. The following is a short outline describing some of the numerous places where your team can raise funds. So don't fret, there are already considerable resources and expertise available, giving your team a great place to build off of and gain the funds and resources your team will need.

Who to Approach?

Just as there are many types of restriction enzymes for cutting DNA, there are many ways you can get prospective supporters to help fund your team. In short, this can include financial grants & donations, scholarships, studentships, in-kind donations of supplies, and promotional rates for services. Most iGEM teams rely on a combination of these sources to cover their budgetary needs within their financial plan each year. Now that we have an idea of the major pieces of the fundraising puzzle, we will discuss each in detail below:

i) Grants – These are often available from major funding agencies in your Province or the Federal government. Each grant can vary significantly in size and scope from a few thousand to tens of thousands of dollars.

   In Alberta, the government is investing in training students in the area of genomics through the geekStarter initiative, run by Alberta Innovates Technology Futures (AITF). Granting opportunities are available for high school teams on a competitive basis, with more information available on their website: http://www.albertatechfutures.ca/AcademicProgramsiCORE/geekStarter.aspx.

   The process typically begins with the submission of an application to the granting agency that describes who your team is and what your team proposes to do with the money. This is usually followed up with a review by the agency, and final decision and if successful awarding of the grant. A few of the major research agencies to which you can apply for grants across Canada or in Alberta are the Natural Sciences and Engineer Research Council (NSERC), Canadian Institute for Health Research (CIHR), Alberta Innovates Health Solutions (AIHS), AITF, and Genome Alberta.

ii) Industrial Grants – Many organizations and industries will support new research and the development of new technologies to solve problems they face.

   These grants may come from specific government or non-governmental agencies whose job it is to support specific industries or to distribute funds raised from that industry. As an example, if your team is developing a system that could solve a specific problem facing the oil and gas industry, then you could target associations within this group such as Canada's Oil Sands Innovation Alliance (COSIA) for support. Another example, if your work is geared towards cattle the cattle industry, approaching the Alberta Livestock and Meat Association (ALMA) could lead to support for your team with both financial and contacts to help you inform the design and development of your system to meet the needs of the industry.

   Many times industrial partners request a detailed breakdown of all funds gathered to date and how you plan to use their funding. Often they have certain restrictions on how funds can be spent - such as travel. The above example budget given above includes a mock application to Genome Alberta - a company that sponsors research geared towards genomics-based solutions to real world problems. The column titled Genome Alberta Request is a detailed breakdown of the use of the funds being requested from the organization.

iii) Donations – Teams have in the past relied on donations from friends, family, businesses and non-profit groups in their surrounding communities. These are usually smaller denomination funds but these donations can rapidly add up.

Many local businesses and nonprofits have specific funds dedicated to supporting local groups in their promising programs in their area. These groups are typically actively looking to give this money away so even just talking the group and asking if they have support available could be all it takes to get a donation. To request donations, teams in the past have put together a one-page sponsorship document that summarizes who they are, what iGEM is, how a potential donation could help the team, and how the team will can recognize the donation.

Other ideas used by iGEM teams in the past to reach out for donations are bake sales, gala style dinners and Science Café like events. Such events can motivate friends, families, and the general public to see how they can support the team, builds public **outreach** opportunities, and gives teams a place to show off what they are doing before the competition.

iv) Scholarships – While not as typical for high school teams, the vast majority of collegiate teams rely on summer studentships to help support their students who work on an iGEM team during the competition season. These scholarships typically are in the range of $4000-$6000 and cover a period of approximately 4 months - during the May to August summer holidays. They can come from major granting agencies (including NSERC, Genome Canada/Alberta, CIHR, AIHS, Markin). Additionally, many Universities also offer their own internal granting scholarships for students attending that institution.

v) In-Kind Donations & Rebates – Many biotechnology companies offer large discounts or free access to their products and services to iGEM teams. Some of these companies have a formal program through iGEM headquarters, such as AutoDesk, MathWorks and IDT. Other companies are simply waiting for you to email them and ask. Major biotechnology suppliers such as BioBasic, Eurofins, GenScript, New England Biolabs, and VWR to name just a few have supported teams in the past with significant donations of materials and supplies to teams.

The Approach

Approaching groups for funding and support will vary significantly between different groups. This will depend on who your team is looking to raise support from. For example, a pitch with a corporate donor to get their support could occur over a single meeting, while approaching a granting agency could involve a thorough application and review process that will be more time consuming. Whereas, approaching friends, family and colleagues could be fast and informal, as routine as a purchase from a bake-sale organized to support your team's travel budget. What has been shown year over year by iGEM teams in both the collegiate and high school realms is that there is no single right way to raising funds for a team.

It is usually very beneficial to dedicate the coordination of fundraising organizational work with to one individual – ideally this would be a student, but will vary depending on each team's internal

structure. A coordinator position will provide continuity and will ensure that all the project's milestones will be met.

A major initial milestone for the fundraising group is to identify who the group is, what they are hoping to accomplish and what funds will be necessary to achieve those goals these can be very rough. By setting a clear long term goal your team can then work to fill in all of the specific details of how that funding will come together. In this case this will require coordination between those budgeting for the experiments to be performed and those raising the funds for the group.

Persistence is Key!

Fundraising can be challenging at first, especially if you have no contacts in place with the suppliers whom you would wish to talk to. Asking for assistance from previous iGEM teams or members of your local university is a good way to start. Never give up! The worst anyone could do is just say no.

9. POLICY AND PRACTICES:

ETHICAL AND SOCIETAL CONSIDERATIONS

Magdalena Pop

1. The Public View and The Role of Scientists

Synthetic biology is a contentious human endeavor. Why? Because it aims to alter and re-program biological systems to serve human purposes, and this poses **ethical dilemmas** and risks. It is these controversial aspects of an iGEM project that must be addressed in Policy & Practices.

To weigh the promises against the perils of your synthetic biology research and to preempt controversy, it helps to ponder the following questions, which are based on moral ideas and norms highlighted in the 2010 report "New Directions – The Ethics of Synthetic Biology and Emerging Technologies" from the US Presidential Commission for the Study of Bioethical Issues.

Does your project advance the public well-being?

Projects must have outcomes that are advantageous to the wider community and that improve people's lives. Proper scientific conduct requires that you strive to promote and advance the public good, while keeping risks and negative side effects to a minimum. To meet this requirement, you must assess the uses and applications of your project against all its possible harmful effects, and you must adjust the course of your project so as to reduce probable risky outcomes as much as you can.

The best way to answer this question calls for involvement of the wider community. Bounce your ideas off members of the public. Share your thoughts, intentions and plans with people from various walks of life, making sure to include in the discussion those who would be impacted directly by your project, either in positive or negative ways. Design your strategy and evaluate the benefits and risks of your project in collaboration with knowledgeable members of the synthetic biology community. All of this will require that information about your project, including your experimental plans and results, is open and easily accessible.

Do you practice responsible stewardship?

To answer this question, you must look beyond the effects that your project may have on the public well-being, and consider its potential implications for those that have no voice and cannot be consulted. Who would those be? Whose welfare could you be held responsible for? Who would depend on your decisions and actions without any possibility for appeal? Those would of course be young children and future generations, as well as all non-human species along with the environment.

This question too calls for a collaborative, balanced and open-minded approach to your project, which could very well aim to change the world as long as it doesn't jeopardize the safety and security of any of the world's inhabitants. Here the long-term benefits and drawbacks of your project need to be evaluated from a wider perspective, with the potential for future harmful developments or malevolent use foremost in the discussion. Precautionary safeguards and methods for monitoring and controlling the undesired spread of any synthetic biology products must be put forth to address all concerns. Even the possibility for unanticipated future developments must be recognized, which demands regular checkups and evaluations.

Does your project foster responsible intellectual exploration?

By their very nature, projects like yours are opportunities to investigate new ideas and to innovate, which forms the basis of democracy and freedom. If out of fear and concern about possible negative developments we would start restricting people's capacity to explore and create, we would be limiting the scientific and technological progress of our society and ultimately the public well-being. But free intellectual exploration comes with obligations, especially towards protecting the safety and security of our communities and of the entire planet.

Do you have a proper understanding of the necessary regulations and restrictions designed to preserve public safety? And do you always abide by those rules and restrictions while conducting your synthetic biology work – with no exceptions? Only by approaching such questions with maximum responsibility and truthfulness will you ensure the accountability necessary for your research and others' to continue unhindered and for risks to be kept at bay.

Do you practice inclusiveness and collaboration?

This question invites you to reflect on how the important decisions are being made during your research. Do you listen to differing opinions and views with an open mind and an unprejudiced perspective? As a young scientific field, synthetic biology faces much uncertainty still. It is therefore imperative that the principles of sharing, cooperation, and transparency are applied faithfully and consistently, even more so than in the established, older sciences. And particularly when discussing opposing views, participants must strive to remain receptive and constructive. Ultimately, the course your project takes must always stress the public good before the interests of individuals.

This being said, while all relevant opinions and perspectives must be considered, effective collaboration and best decision making occurs when all those engaged have an appropriate scientific and ethical background. Has every effort been made to provide everyone involved, inside or outside the team, with accurate information and the education required to form valid opinions and to make sound decisions? Helping as many people as possible grow their competency in synthetic biology and inform their views on it will undoubtedly be one of your most prominent accomplishments.

Does your project encourage fairness and justice?

It would be safe to assume that your synthetic biology project will create a solution to a problem, and also that this solution will be made available to the public. But how will you ensure that those who are most likely to benefit from this solution will have ready access to it? As scientists, we have a moral duty to share the positive outcome of our research in an honest and indiscriminate fashion. And at the same time we should strive for fairness in dealing with its drawbacks and negative effects.

To be fair and just, you must think about how to maximize the benefits by helping those in highest need reach the end product of your project. But also think about ways to manage the risks so that they do not impact anyone or anything more than absolutely necessary.

2. Ethics in Science – From Past to Present

Like any major human activity, science has numerous implications for society, some of which interfere with **moral values** and codes. Indeed, from the remote past to the present day, there have been multiple occasions when humanity was faced with ethical conundrums involving science and technology practices. Therefore, the principles outlined above have deep and complex roots in the history of human society and in the evolution of democracy and civilization.

It is well known that during the Middle Ages church authorities tried to hinder any scientific exploration that was perceived as a threat to religion. Ideas and discoveries that contradicted the church's doctrine were often rejected as heresies. Very few members of the public possessed the education and information necessary to grasp the new ideas and to decide for themselves if they were valid or not. Centuries later, as the Industrial Revolution unfolded, **intellectual freedom** and the access to education improved, which helped the sciences advance at an unprecedented pace. In

fact, during the 19th and the first half of the 20th century, scientific discoveries and technological capabilities were growing almost completely unhindered by any ethical rules at all. Moral concepts such as accountability, fairness, or responsible stewardship were given the backseat if they were considered at all. This is why some of the greatest scientific advances of all times, which took place during the 19th and early 20th century, ended up being used not only for beneficial purposes but for destructive ones as well.

For example, the mechanism of **heredity** and Darwin's **theory of evolution by natural selection** – two cornerstones of the life sciences, both laid in the second half of the 19th century – provided the basis for **eugenics**, a doctrine that brought immeasurable harm around the world. Eugenics was used to justify social policies and interventions that discriminated against members of the population who were considered inferior, or "unfit", e.g. the Jews in the view of the Nazi. Needless to say that this perversion of science was extremely immoral and evil! A similar example occurred in the field of atomic and nuclear physics. At the end of the 19th century and the beginning of the 20th century this scientific field was revolutionized as physicists uncovered the structure of matter and the inner workings of matter's building blocks, the atoms. Although we owe many of our comforts today to these discoveries – think light, electronics, or energy sources – we must remember that they also paved the way to nuclear weapons and the atomic bomb, which had devastating consequences on Japan at the end of WWII.

Present-day science and technology are no different in that they expose us to many ethical dilemmas and traps. The recently revealed use of Internet technology for massive surveillance purposes has opened a new debate around issues of democracy, freedom, and justice. Genetic engineering and synthetic biology too are being debated and scrutinized, as they are still vulnerable to plenty of mishaps intended or otherwise. The creation of the first fully synthetic bacterial **genome** in 2010 was a like a wakeup call. Public authorities responded to the announcement by pondering new regulations needed to guide and monitor synthetic biology activities, which became possible to conduct in ordinary people's homes. The realization of the potential that synthetic biology has, both for good and for evil purposes, called for urgent and responsible vigilance. An organization based in Europe and concerned with best practices in synthetic biology was created: the International Association for Synthetic Biology (IASB). In the US, the Federal Bureau of Investigation (FBI) has designated special resources to promoting responsibility in scientific research, and to guide and oversee synthetic biology activities with the aim of preserving the safety and security of the population and preventing the production of bioweapons. As sponsor of the iGEM competition, and in particular of the initiatives involving **biosecurity** topics, the FBI is reaching out to young researchers and encourages openness and cooperation.

In February 2014 biosecurity risks associated with synthetic biology were addressed anew in a workshop held at King's College London. The main goal of the workshop was to assess how real and serious these risks were, and to discuss methods necessary to mitigate them. The report that ensued reflects the complexity of the topic as viewed from a societal perspective. Public perception of threat from synthetic biology, the report says, is heightened both by the way in which the field is covered by the media, and by synthetic biologists' own claim of making biology easier to engineer. On one hand, blown up representations in the media are somewhat unavoidable, and there is usually value to them. Provided that possible yet improbable scary scenarios aren't presented as scientific realities, it's safer to consider rather than to dismiss them. On the other hand, emphasizing the engineering nature of synthetic biology is more avoidable, in light of the public misconception of an engineering discipline as somehow more accessible to the layperson, and requiring of less skill, technology, and expertise. Indeed, although some aspects of the work involved are becoming easier, there are and will continue to be plenty of requirements for specialist knowledge and skill which are not easy to learn and transfer. This conclusion has reassuring connotations for the embattled field of synthetic biology. In its analysis, the report also identifies prominent biases that each of the groups involved– synthetic biologists, policy makers, social scientists, environmentalists - brings to the table, and emphasizes the need for increased collaboration among these groups whereby everybody should strive to move beyond defending their own opinion and toward finding a common ground.

3. Regulating Synthetic Biology - Legislation and Policy Aspects

Synthetic biology is advancing so fast that policy makers and regulatory organizations struggle to keep up. Not that there aren't any rules! Because it extended from **recombinant DNA technology** and genetic engineering - which have already been around for a few decades – synthetic biology complies with policies and laws that are already in place to address risks and hazards associated with genetically engineered organisms. But how effective are these regulations? The more complex and diverse the applications of synthetic biology and their implications become, the more discussions around this topic intensify.

How does the current regulatory system work, and what will need to change? Even though it's grounded on common principles and ideas, the regulation of biotechnology, and in particular of genetic engineering, differs between countries. The iGEM foundation provides a list of web links to regulatory systems from different parts of the world. Most of these systems are being reevaluated and revised to keep up with current trends and developments. Here is the web link to the newest Canadian Biotechnology Strategy. As in other parts of the world, in Canada too it is recognized that biotech regulation must become more effective in dealing with emerging complexities in the field. In US, this recent report contains similar findings and recommendations. Synthetic biology, the report says, has far greater capabilities to engineer organisms than previous techniques allowed. This will result in a new generation of engineered organisms, which will not only be larger but also a lot more varied than the existing one. In keeping up with these changes, policy makers need to consider the following aspects:

- Some of the newly developed synthetic biology methods and products are too unconventional to be covered by the existing regulatory procedures, and therefore may reach the market without proper assessment and review. Safety risks in particular will need to be given a lot more attention in light of how elaborate and complex the new systems have become. Present testing and assessment procedures focus on applicability and economic impact, but this will need to change. The **evaluation of short- and long-term safety risks** must occupy a more prominent place in the future.

- The sheer number and variety of engineered organisms makes the reviewing and regulatory process increasingly challenging. Product-by-product pre-market review, as demanded by current procedures, will soon become overwhelming and unfeasible. This calls not only for **making policy more versatile**, but also for **adjusting the regulatory infrastructure and increasing support in funds and expertise**.

- The multidisciplinary and complex nature of synthetic biology demands **a rethinking of how information and results are owned and shared**. The traditional patent-oriented approach, with an emphasis on confidentiality, proves inadequate for such a dynamic and highly scrutinized field. What would help instead would be **policies that promote open-sourcing, transparency and self-regulation**. These would be beneficial because they would not only allow the public to participate in debating and decision making, but to share responsibility too.

4. Outreach and Policy & Practices

As discussed above, the Policy & Practices component of an iGEM project should take a critical look at the benefits and costs of the project in terms of its ethical and societal consequences. This project

component often includes **outreach**, where team members engage the public in various ways, such as school visits and information sessions, or tours of the laboratory in which their iGEM team works. Although outreach is an important part of iGEM and of being a scientist, it only serves a limited goal and therefore shouldn't be the only component of Policy & Practices. Through outreach teams promote science and technology, and spread the word about iGEM and their project, but do not necessarily analyze the merit of their project through a critical lens with a focus on ethics and the project's costs and benefits to society. These latter aspects are at least as important as the outreach, and must be addressed as well. Below are some excellent examples of Outreach and of Policy & Practices (aka **Human Practices**) project components.

Outreach

- ArtSciBangalore: http://2011.igem.org/Team:ArtScienceBangalore/Outreachhttp://2011.igem.org/Team:ArtScienceBangalore/Outreach
- Calgary: http://2012.igem.org/Team:Calgary/Outreachhttp://2012.igem.org/Team:Calgary/Outreach http://2012.igem.org/Team:Calgary/Outreach

Human Practices

- Manchester http://2013.igem.org/Team:Manchester/ManagementPart1http://2013.igem.org/Team:Manchester/ManagementPart1
- NYCWetware: http://2011.igem.org/Team:NYC_Wetware/Food_Practiceshttp://2011.igem.org/Team:NYC_Wetware/Food_Practices
- Lethbridge: http://2010.igem.org/Team:Lethbridge/Ethics

References:

1. New Directions – The Ethics of Synthetic Biology and Emerging Technologies (Presidential Commission for the Study of Bioethical Issues, December 2010) http://bioethics.gov/sites/default/files/PCSBI-Synthetic-Biology-Report-12.16.10_0.pdf
2. The NSA Files - The Guardian http://www.theguardian.com/world/the-nsa-files
3. Researchers start up cell with synthetic genome (Alla Katsnelson, Nature online, 20 May 2010) http://www.nature.com/news/2010/100520/full/news.2010.253.html
4. Workshop Report: Synthetic Biology and Biosecurity: How Scared Should We Be? (Catherine Jefferson, Filippa Lentzos and Claire Marris, King's College London, May 2014) http://www.kcl.ac.uk/sspp/departments/sshm/research/Research-Labs/CSynBI@KCL-PDFs/Jefferson-et-al-(2014)-Synthetic-Biology-and-Biosecurity.pdf
5. Synthetic Biology and the US Biotechnology Regulatory System: Challenges and Options (Sarah R. Carter et. al, May 2014) http://www.jcvi.org/cms/fileadmin/site/research/projects/synthetic-biology-and-the-us-regulatory-system/full-report.pdf
6. Time to settle the synthetic controversy (Volker ter Meulen, Nature, 7 May 2014) http://www.nature.com/news/time-to-settle-the-synthetic-controversy-1.15169
7. When and How Will We Regulate Synthetic Biology? (Pete Shanks, Center for Genetics and Society, 6 June 2014) http://www.geneticsandsociety.org/article.php?id=7806
8. Safety at iGEM http://igem.org/Safety

9. Canada's Biotechnology Strategy http://www.hc-sc.gc.ca/sr-sr/biotech/role/strateg-eng.php

10. THE WIKI

Patrick Wu

1. The Wiki

What is a **wiki**? Why is it so important? This chapter will cover the basics of creating your team's wiki and hopefully provide some stepping stones for you to create the Best Wiki at the iGEM competition. We will cover some basic concepts in designing a good wiki, and I will describe HTML and CSS techniques you can use to help circumvent the iGEM wiki's default behaviours.

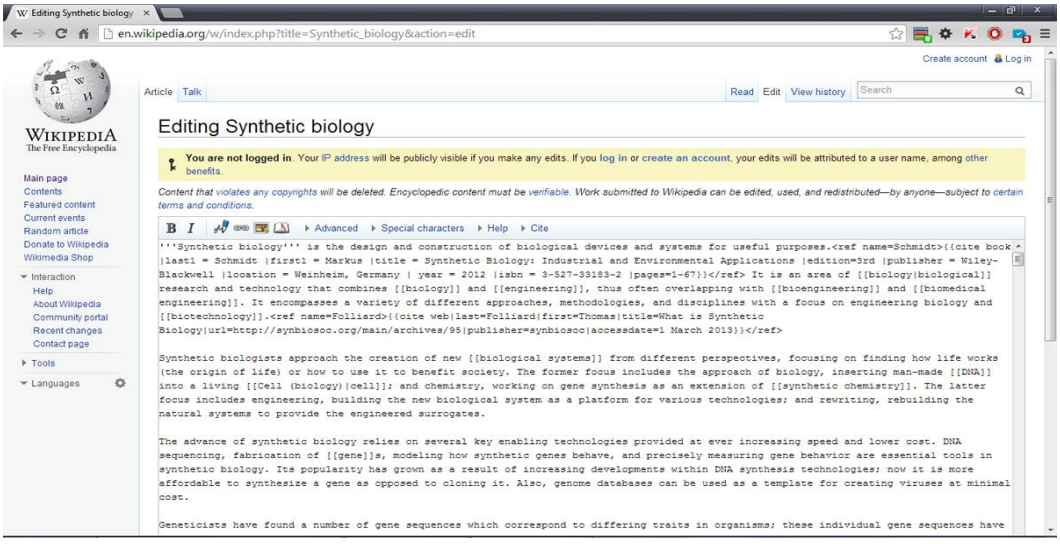
The term *wiki* was coined by Ward Cunningham, the inventor of the first wiki, and comes from a Hawaiian word meaning "quick". A wiki is a tool that allows **collaborative edits from multiple people** on a website. This means any member of the team is able to add, modify, or delete content as they see fit. This is ideal for the iGEM competition because this allows the entire team to contribute data to the site without burdening a small team of web developers. (The developers have a lot of work of their own to worry about, as you'll soon see!)

Arguably the most popular wiki is Wikipedia, where you can see this kind of collaboration in action. Many contributors can modify information on any page of Wikipedia. On a smaller scale, this is what your team will be doing, too.

If you have ever edited content in Wikipedia, you may notice another feature of wikis. *Wiki markup* is the simplified language used to add content to pages. The iGEM wikis run off a similar engine (MediaWiki) and you can use similar code in order to edit pages on the iGEM wikis.

Syntax refers to the "grammar" that lets the browser **unambiguously** understand what you want it to display. For example, in wiki markup, square brackets are used to denote links to other pages within the same wiki.

The iGEM wiki is capable of interpreting HTML and CSS syntax, which is a much more powerful set of languages that allows finer and more sophisticated control over the design of your wiki. Wiki markup is a simplified template that is translated into HTML when your browser loads the page, so anything



Wikipedia is a very well-known wiki. The markup used has special formatting to denote features such as links, images, tables, and bolded, italicized, or underlined text.

wiki markup can do can theoretically be accomplished with HTML and CSS. Nowadays, almost all Collegiate-level teams will have their wikis coded in HTML and CSS, and forgoes wiki markup almost entirely (with a few exceptions). While more complicated, learning HTML and CSS will ultimately give you greater rewards as you will have much better control over what you are building.

2. Why Should I Care About the Wiki?

The simple answer is because the wiki is a powerful tool that allows you to communicate what your project is to the judges, other iGEMmers, and the rest of the world. Judges at the competition are very busy people who have to watch a large number of presentations throughout the day. The wiki is your opportunity to remind the judges what your project is, and to highlight what your major accomplishments are.

Remember that the judges aren't necessarily scientists trained in **synthetic biology**. It's recommended that you write the wiki so "anybody with a high school level of education" can understand it. In other words, you should be writing your wiki so that if your friends and family read it, they will understand the basic idea behind your project. Therefore, try to avoid using too much scientific jargon, and if you do, make sure you explain what it means.

This is your opportunity to show off everything you've learned over the summer not only to the judges, but also your family, friends, and the rest of the world.

Also, your wiki is being judged along with your project, and there is an award for Best Wiki at the competition. You can keep that in mind, if you wish.

3. What's in a Wiki?

The official requirements about what can and cannot be put on the wiki can be found here: http://2014hs.igem.org/Requirements/Wiki. There should be a similar page each year (as of this writing, the 2015 site does not have guidelines just yet). In summary, these are your main requirements:

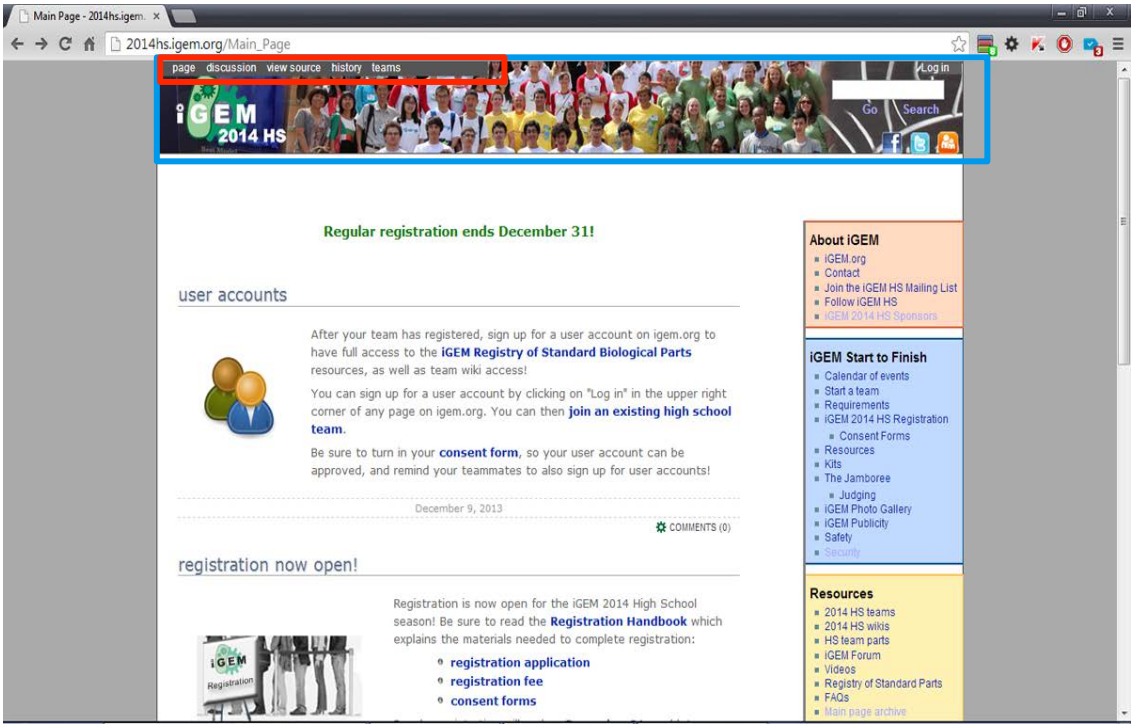
- **You must have your project documented by the deadlines** – there are a few major deadlines that are outlined on iGEM's Calendar of Events. One of your most critical dates is the **wiki freeze**, where you must have everything on your wiki finalized before the servers are locked down. After the wiki freeze, you will not be able to make any more changes to your wiki until after the iGEM competition. You should be working on your wiki throughout the competition, but be ready to set aside a few days prior to the wiki freeze deadline to polish up any last details.
- **All content must be uploaded to the iGEM server of your respective year** – there are a number of different domains that iGEM runs off of. Make sure you upload all your photos, images, and wiki-building content (like buttons and icons) on the proper server: *20XXhs.igem.org*, where *20XX* is the year of your competition
    - **Do not** confuse *20XX**hs**.igem.org* with *20XX.igem.org* since the latter is the Collegiate server of the same year!
- **Flash is not permitted** – with the current trends in tablets and smartphones, this shouldn't be a major loss. Adobe Flash, while it produces very visually appealing sites, is not a good crutch to be building a website on. Also, iGEM strives to make all wiki code open-source (in other words, you are free to take any code from any wiki to use on your own—as long as you credit them, of course.) Flash hides this code into an embedded movie and therefore goes against this open-source philosophy.
- **Your wiki must remain within your own namespace** – for example, if your team is called *Example_Canada*, your homepage on the iGEM servers will be on

20XXhs.igem.org/Team:Example_Canada.

This is your *namespace*. Any new pages you create must have *Team:Example_Canada* before it. For example:

20XXhs.igem.org/Team:Example_Canada/Team
20XXhs.igem.org/Team:Example_Canada/Project
20XXhs.igem.org/Team:Example_Canada/Project/SubProject

- **Your wiki must contain a safety and attributions page** – the Safety page is a questionnaire that you must answer in order to demonstrate to iGEM and the judges that you are protecting yourselves and others as you are working over the year. The safety page also allows you to demonstrate a thorough understanding of the potential safety issues your project might create. How are you handling them? How are you mitigating risk? The attributions page is important for showing that this project is done primarily by yourselves as students (as opposed to having a teacher or university mentor doing all the work for you). The attributions page must list any external help you have received, such as labs which have given you materials, web designers that have designed the wiki for you, and so forth.
- **You must have a link back to the iGEM home page** – this is an iGEM competition, after all. By default, you have a large photographic banner at the top of your wiki which is sufficient to lead viewers to iGEM's main site. However, if you find this banner rather unsightly and decide to remove it through your code, you must provide a link back to iGEM's site somewhere else. **You cannot erase the top control which lets you edit pages and log in**. But iGEM teams in the past have pieced together CSS code which can erase the photo banner while preserving the control bar. You can find a code snippet in Appendix A.



The top banner (blue) appears by default on all iGEM pages, and links to the main iGEM page. The menu area (red) only appears if you roll your mouse cursor over it. It does not easily appear on touch-based devices such as tablets. The CSS snippet provided in Appendix A removes the banner but preserves the menu. The snippet also removes the mouseover behaviour of the menu and instead keeps it visible at all times.

Some other key components of the wiki include:

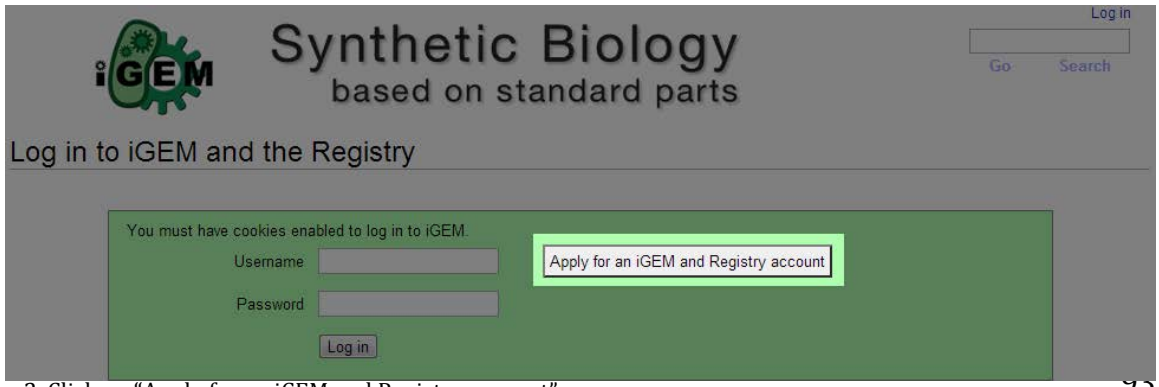
- A **project page** –this is where the description of everything you have done over the year will be found. Be sure to explain what the problem you were tackling was, and what kind of solution you had proposed. What were your experiments? Why did you do them? What were you hoping to see, and do your results reflect this? What can you conclude from your results? *Can* you conclude something from your results (incidentally, something that is very common in science and should not be considered a failure)? What needs to be done in the future? This will likely be the largest section of your wiki, so you should be ready to break this into a number of different pages (such as your introduction, methods, results, and conclusions).
- A **notebook page** – this is where you document the day-to-day experiments that you have done. This is critical in science, as this allows peers to review your experiments and your thought processes. You do not need to repetitively mention the amount of reagents that you're using, but you should be mentioning which parts you are using and what constructs you are trying to build. The 2013 Lethbridge high school team (http://2013hs.igem.org/Team:Lethbridge_Canada) has a good example of a notebook (and of a wiki in general).
- A **team page** – who are you? This section lets you briefly talk about yourselves as students. This page can be as simple or as elaborate as you want it to be. Take a look at other iGEM teams for inspiration.
- A **protocols section** – a lot of teams place this within the Notebook section of their wiki. This is a summary of all the molecular biology techniques and experiments you have done over the year. Others who read these protocols should be able to replicate your experiments and get similar results.
- A **policy and practices section** – this is where you discuss your **Policy and Practices** component of the project.
- A **sponsors section** – thank the people who have helped you along the way! You can also put your required attributions here.

By no means is this list exhaustive. Feel free to add or omit any sections you feel are necessary in order to get the best parts of your project across to the judges.

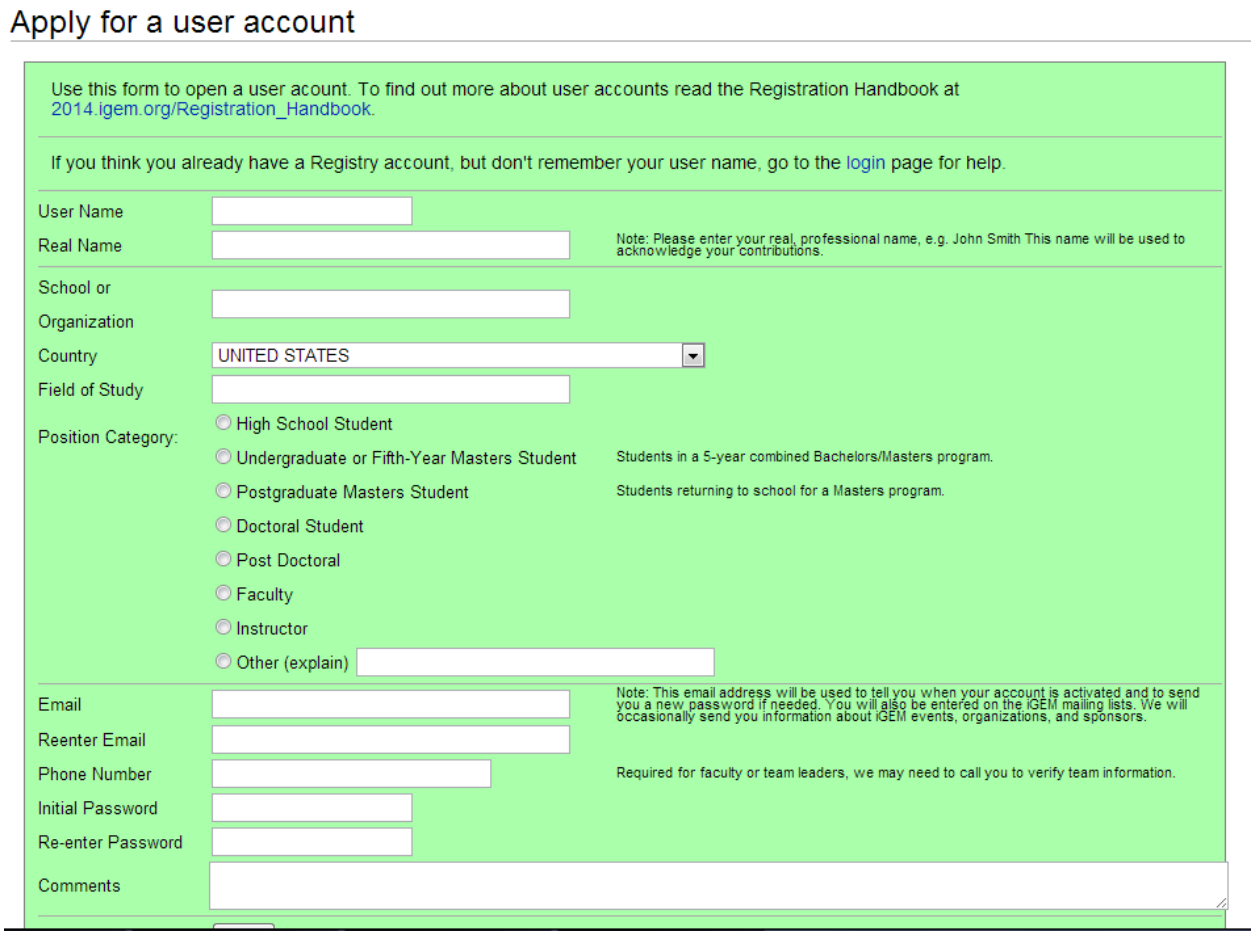
4. Accessing the iGEM Wiki

Once your team is signed up and registered in the High School competition, you will be asked to create an account with iGEM. This is what will allow you to edit the wiki.

1. Click on "Log in" on the top-right hand corner of the iGEM banner.

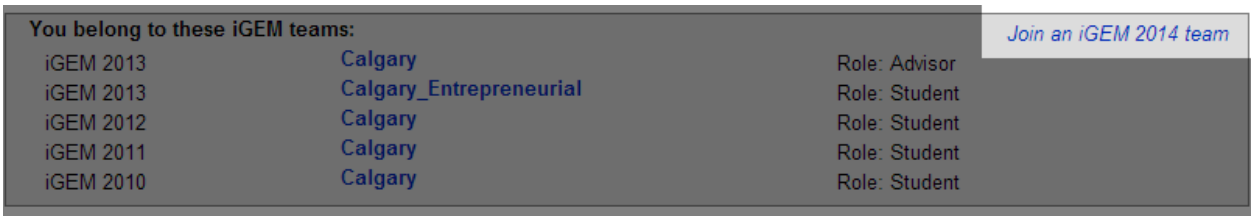


2. Click on "Apply for an iGEM and Registry account".



3. Fill in your information and click "Apply"

4. After logging in, click "My account" in the upper left-hand corner

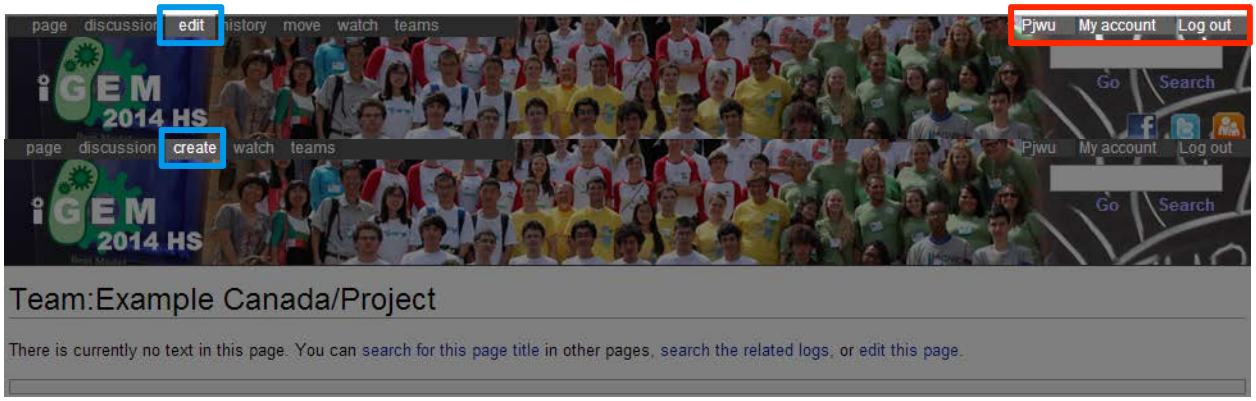


5. There will be a section which lists all of the teams you participate in. As a new user, you will not have anything here. Click "Join an iGEM 2014 team" and follow the instructions. Your team leader will add you to the roster.

Make sure your instructors/advisors have added you to the proper team. You will not be able to edit any wikis on the High School server until you are part of a team.

5. Editing the Wiki

Editing the wiki is as simple as logging in and clicking "Edit" in the menu at the top of the banner. If you see "View source" instead, you have not logged in properly or the iGEM servers have kicked you off. Try to log in again.



For example, if the page to be created is a project page in the namespace *Example_Canada*, you would first navigate to *http://2014hs.igem.org/Example_Canada/Project*. You should then see this message. Click "Create" to make a page.

To create a new page, type the URL of the future page into the address box. You should see a message telling you that this page does not exist. Click "Create" on the top menu and you will be brought to the editing window.

From here, you can type all of your code into the box and then click either "Show preview" to see how it impacts the final page or "Save page" to commit it to public viewing. Remember that in order to have changes visible to others, you must **save** the page. However, it is a good habit to **preview** pages first, in order to confirm you have not made any flagrant mistakes prior to publishing the page for the public to see.

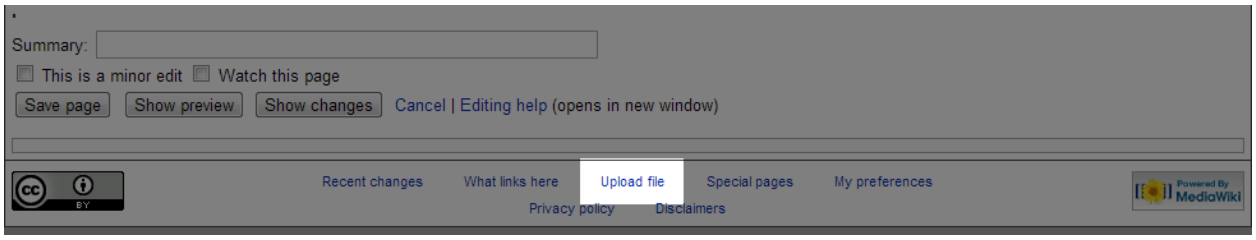
The large white area is where markup will go. The "Save page" and "Show preview" buttons are near the bottom (blue).

Feel free to play around with a wiki page on your own namespace by clicking "Edit" at the top of the banner (preferably a page within your own namespace to minimize damage). Historically I have always liked to create a page called *Sandbox* in the namespace so that I can test out code without the public seeing any changes until it is fully done. Then I would copy and paste the markup onto its destination page. It is good practice to build pages elsewhere on the wiki and avoid publicly launching a wiki page until you are comfortable with how it looks. This also saves the viewers from trying to work with a buggy wiki.

6. Uploading and Using Images (and Other Media)

In order to build a successful wiki, you will need to be able to upload images on to the iGEM server so that you can utilize them in your wiki. Remember that one of the official rules for the wiki is that all content must be hosted on the wiki server of that year.

At the bottom of the page (if you are logged in), click the "Upload file" link. If you cannot see it, ensure that you are logged in and that the iGEM server has not kicked you off.



The "Upload file" link only appears if you are logged in.

Choose a file by clicking on the "Choose file" button (blue). Then, give the file a name which will be used to find the file again on the server (red). Finally, upload the file by clicking the "Upload file" button (purple). The destination filename cannot have spaces, but can contain underscore characters (_) to represent spaces.

The remaining steps should be intuitive. The only point I must stress is that **all images share the same namespace** on the iGEM server, meaning any image your team uploads can be overwritten by another team if they upload a file of the same name. Therefore, **you cannot upload an image with simple or generic names like "Image1.jpg"** because there is a high risk that somebody on a different team can overwrite your photo by uploading their own version of "Image1.jpg".

My recommendation is that you prefix the file with something strictly for your own team, such as *LethbridgeHS2014_* or *ConsortHS2014_* so that the file is less likely to be mistaken for another team's file.

After you click "Upload file", you will be brought to a screen which will describe details about the file you have just uploaded.

There are three ways to find this image you have just uploaded. You can find it again either through the URL (red), the wiki server filename derived from the URL (blue), and the direct link to the image you have uploaded (if you click the link in purple). The browser URL on this page cannot be used for HTML and CSS purposes. Only the direct link can be used for HTML and CSS markup.

The wiki server filename is only needed when dealing with wiki markup. The direct URL link provides a link to the image for HTML coding purposes. You will require that if you decide to use <img> tags since wiki markup cannot be used in HTML and CSS.

In summary, you have the browser URL, the wiki server filename, and the direct URL to refer to this image you have uploaded. **Ensure that you keep and save at least one of these addresses so you are able to find this file again if need be.**

7. User-Centered Design and User Experience Design

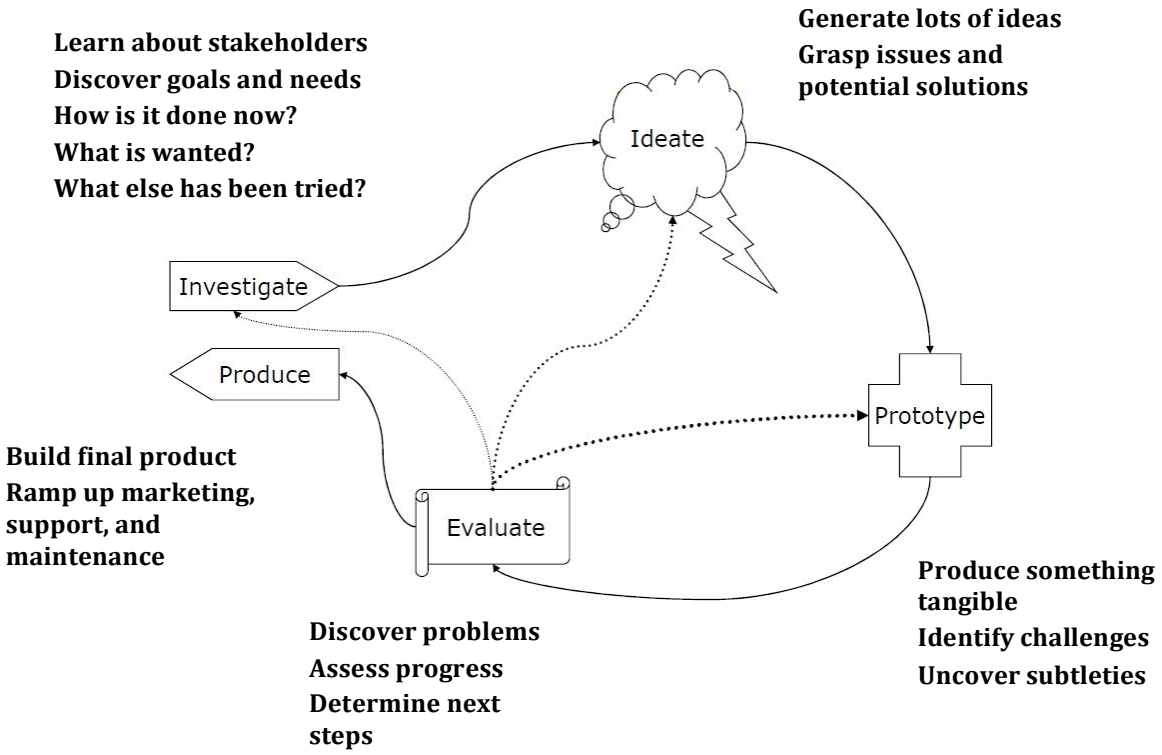
Think about the websites, apps, and computer programs that you use often. Do you enjoy using them? Do you find some features on some of them rather frustrating? Do you find yourself lost when you're using some apps? Keep these questions in mind as you go about your daily web browsing today.

There is plenty of very careful thought that is put behind designing a good user interface. The process behind good design is known as **user-centered design (UCD)**, and usually well-designed interfaces have many hours, weeks, or even months of design, prototyping, and testing behind them. UCD is part

of a much larger field of **user experience design (UX)**. UX is in turn part of **human-computer interaction (HCI)**, a field of computer science that intersects graphic and industrial design, behavioural sciences, and communication theory, amongst many others. Many papers and journals for UX exist, and it continues to be a very heavily researched field.

UCD is an iterative process that focuses on building an interface that facilitates the needs and desires of the **user**—in this case, whoever will be viewing your wiki. UCD cycles through four main steps:

- **Investigating** what the user does and wants to have
- **Ideating** or brainstorming ideas that can be used to solve the problem at hand
- **Prototyping** a few of these ideas and develop them into potential products, and
- **Evaluating** the prototypes with the users to see if they are effective and usable solutions



The UCD cycle begins with investigation and proceeds to ideation, prototyping, and evaluation. Note that traditionally, if something fails in any of the latter steps, there is a path which leads back to earlier steps and the cycle repeats itself. Only when the evaluation succeeds does the product go into production.

Why is this relevant? UX is about making the users **happy** and **satisfied** with using the interface. The easier you make it for the user (namely, the judges) to access all the information you have on your wiki, and the more pleasant the experience is, the less frustrated the judges will be with you. And in a competition like iGEM, annoying the judges is likely not high on your list of priorities.

UX and design in general is difficult—there wouldn't be Ph.D. degrees in those fields otherwise—but you are taking a major step by simply considering who might be using this interface/product in the natural conditions that they will be using them. For example, will the users be viewing your wiki on

laptops? (The answer is "yes"). Are they going to be viewing them on tablets? (The answer is also "yes"). Are they going to view them on phones? (The answer is… well, you know.)

What does this imply? What do you need to do in order to make it easier for these users? (Hint: tablets do not have a mouse-over action. All you have is a thumb that taps. Try to edit the wiki on an iPad, for example, and you will find it rather difficult to access the mouse-over control bar on the default templates.)

As you're building your wiki, or as you're drawing and sketching your ideas for potential interfaces and making prototypes, let other members of your team test them out. As the designer, **you know your interface too intimately to be unbiased** about how to use it, so your judgment alone is not necessarily the best judgment. Watch as your user tries to navigate your interface. Don't help them. Note where they seem to be confused or struggling, and note where they are easily finding the information they need. Use this information to build on new iterations of your idea. Be prepared to make alterations to your interface as necessary in order to accommodate for usability challenges that people find.

8. Nielsen's Usability Heuristics

One of the more well-known individuals in web usability is Dr. Jakob Nielsen, one of the co-founders of the Nielsen Norman Group and has been researching and consulting in web usability since 1998. Nielsen has a Ph.D. in human-computer interaction and has published many articles, patents, and books on how to improve web usability.

Heuristics are general principles, or rules of thumb. For interaction design, Nielsen published ten particular usability heuristics, which can be found on the Nielsen Norman Group website (http://www.nngroup.com/articles/ten-usability-heuristics/). Each one will be covered in some detail here, but remember that these are general guidelines and may not necessarily apply to all situations:

- **Visibility of system status—***The system should always keep users informed about what is going on, through appropriate feedback within reasonable time.*

  If you have ever installed a program before, you will have likely seen small animations like a spinning wheel or a progress bar. These help the user recognize that the computer is, in fact, doing something with the information provided to it. Providing feedback ensures the user doesn't get too lost in what the system is doing.
  On a website, this can even be as simple as highlighting the section of the site on the navigation menu. This way, users can remember what part of the website they are reading. Here's a simple tutorial: http://hicksdesign.co.uk/journal/highlighting-current-page-with-css
- **Match between system and the real world—***The system should speak the users' language, with words, phrases and concepts familiar to the user, rather than system-oriented terms. Follow real-world conventions, making information appear in a natural and logical order.*

  Say you were using a desktop application and suddenly this appeared:

  ```
  A runtime error has occurred.
  0x49cfb8
  Error: Syntax error.
  Do you wish to debug? Y/N
  ```

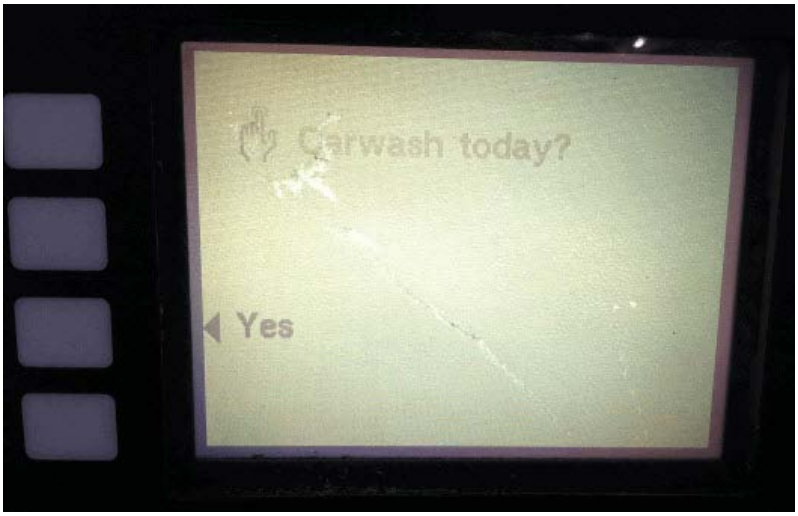
  Does an error message like that mean anything to you as a user? Likely not, since a message

like that was more intended for the developers. These system-oriented words are meaningful only to a small subset of the end users.

In web design, there are particular words that people are used to seeing and have an implied meaning. “Home” brings the user to the front page of the site. Esoteric naming of links confuses users (one example being the official website for the movie *Space Jam*—a site that incidentally never left the 90’s for many other reasons). For iGEM, judges understand terms such as “Notebook”, “Safety”, and “Attributions”, and have expectations as to what can be found on those sorts of pages. Therefore, we recommend that you keep pages with those names.

- **User control and freedom—***Users often choose system functions by mistake and will need a clearly marked "emergency exit" to leave the unwanted state without having to go through an extended dialogue. Support undo and redo.*

  In essence, allow escape routes so that users do not feel trapped when going through a process. You should offer a way out (even something like a red X on the top corner of the window to abort the whole process is still considered a way out).
  People learn interfaces by exploring. Exploring means to poke around the interface to see



There is no escape.

what particular actions do. People make mistakes, so you shouldn’t punish them for attempting to do something. For example, shopping websites will let you review your information before finally committing to a payment. In the worst case, many websites have a “Home” button that simply brings the user back to a safe, reset area where they can try to explore again.

- **Consistency and standards—***Users should not have to wonder whether different words, situations, or actions mean the same thing. Follow platform conventions.*

  It may sound really nitpicky and almost unnecessary, but consistency in fonts, colours, shapes, and wording reduces the chance for users to be surprised. Having *internal consistency* means that an interface is consistent with itself, such as having system buttons collected together. *External consistency* is where the interface is consistent with similar applications on similar platforms. This is where “muscle memory” problems between different programs can cause frustrations. For example, one of Adobe Illustrator’s undo functions (where one can undo multiple steps) has Ctrl+Z as its shortcut, while Adobe Photoshop’s same undo function is Ctrl+Alt+Z. This is where external consistency breaks

down.

In the wiki's case, this rule overlaps with using real-world words. Again, judges expect to see a Notebook section of the wiki, since it is conventional for all teams to have one. However, other website conventions exist, too. For example, you expect to have a navigation menu somewhere on the top or on the side. It would be rather unconventional and confusing to have the navigation menu at the bottom of the page. Take time to make sure your images and text are aligned and well-organized. An uncluttered website is much more pleasant to navigate than a disorganized mess.

- **Error prevention—***Even better than good error messages is a careful design which prevents a problem from occurring in the first place. Either eliminate error-prone conditions or check for them and present users with a confirmation option before they commit to the action.*

  This is a good opportunity to watch your users test out a prototype. Where do they make mistakes? What can you do to help reduce the chances of that mistake happening again? Many desktop applications have menu items on dropdowns that are greyed-out in order to prevent users from performing actions that are impossible.

  When building your wiki, be sure to test out all the links and all potential areas where you think an error can happen. It would be tragic post-wiki freeze to realize one of your major links doesn't actually lead to a page you wanted it to because somebody had copy-pasted the code from another page.

- **Recognition rather than recall—***Minimize the user's memory load by making objects, actions, and options visible. The user should not have to remember information from one part of the dialogue to another. Instructions for use of the system should be visible or easily retrievable whenever appropriate.*

  Recognition is in general much easier for people than recall. For example, if I show you this image, you can immediately recognize what it is:

"It's a jackal, right?"

You may have a harder time with *recall*, for example, if I ask you what colour the shirt you wore was two weeks ago. In general, recognition of an item is much easier than having the user attempt to recall the information for themselves.

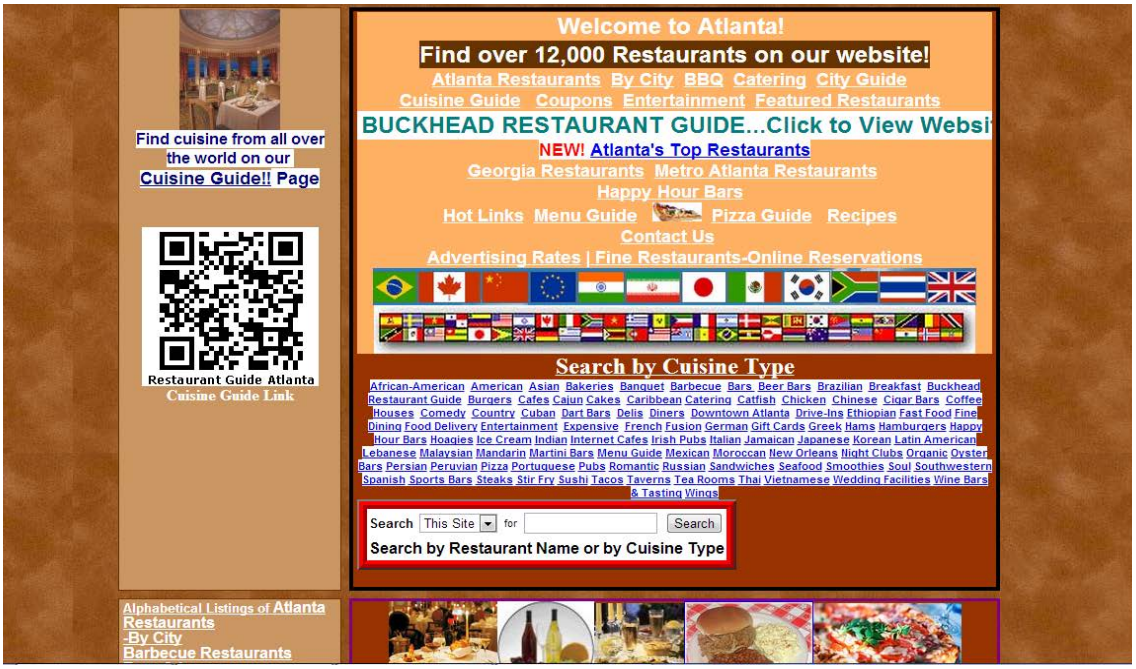
As another example, is a multiple-choice exam easier than a short-answer exam? Sometimes you can pick out what you were looking for when the option is presented to you. It is generally easier to pick an item out from a list instead of having to write it out yourself, because seeing that item in the list can help remind you of what the choice is.

- **Flexibility and efficiency of use—**Accelerators – *unseen by the novice user – may often speed up the interaction for the expert user such that the system can cater to both inexperienced and experienced users. Allow users to tailor frequent actions.*

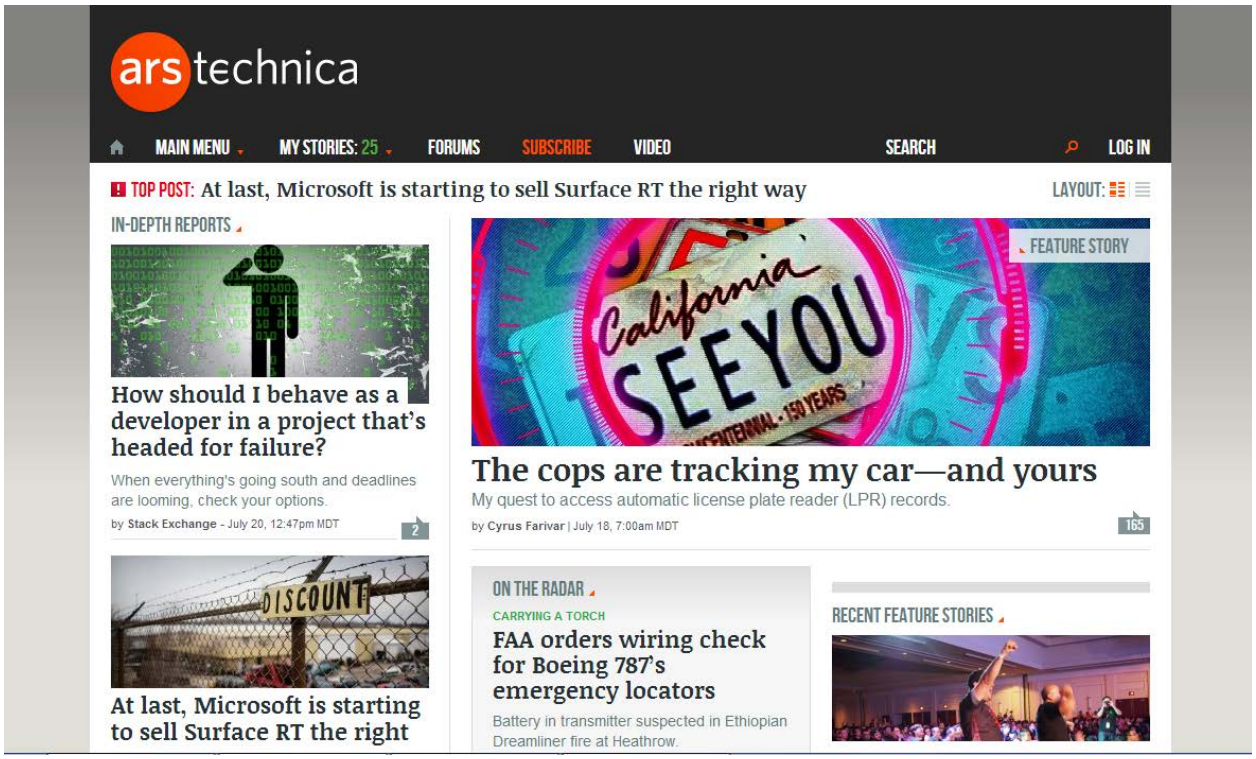
  Accelerators are essentially shortcuts that optimize your workflow. Do you right-click items to copy and paste? Or do you use the Ctrl+C and Ctrl+V shortcuts on your keyboard? (Or ⌘+C and ⌘+V for you Mac users?) Do you hit "Enter" when you submit a form, or are you forced by the interface to click "submit"? This is more commonly a consideration for more complex web applications such as email or shopping sites. One easy way to improve efficiency is to add hyperlinks in your main text. If you're talking about another aspect of a project in one of your paragraphs, provide a link to it so the user can simply jump to that aspect of your project and then return later. Keeping your site structure simple will be a major step in preventing users from getting lost. The general rule of thumb is that all pages should be accessible within three to four clicks of the home page (but this has been debated: http://uxmyths.com/post/654026581/myth-all-pages-should-be-accessible-in-3-clicks)

- **Aesthetic and minimalist design—***Dialogues should not contain information which is irrelevant or rarely needed. Every extra unit of information in a dialogue competes with the relevant units of information and diminishes their relative visibility.*

  This concept was popularized by a number of technology companies in recent history. Apple, Google, and Microsoft have interface design languages. Content is king, and should be brought to the forefront. Keeping your design clutter-free helps users find the information they do need in an easy manner.



Clutter prevents people from finding what they want to find. Whitespace can be more effectively utilized (along with a substantial number of colour and font changes) in order to space out and delineate sections better.



Minimalist design doesn't mean minimal content. Sites such as ArsTechnica are very effective at presenting a large amount of information in a clean, organized manner. Note how whitespace is utilized to prevent text and images from being too close to each other.

However, you should be cognizant of the concept of minimalism even as you are writing content for your wiki. How can you concisely convey everything you need to tell your audience without losing important information? Are you rambling? Can you cut this paragraph down and express yourself more concisely?

Minimalism is deceptively difficult. If too much is removed, the wiki may look incomplete or bland. There is a fine line that separates a clean wiki from a barren one. This is where you will need to exercise some graphic design and artistic skill.

- **Help users recognize, diagnose, and recover from errors—***Error messages should be expressed in plain language (no codes), precisely indicate the problem, and constructively suggest a solution.*

It's not always sufficient to provide error messages such as "An error has occurred" since that does not give information to the user about how to fix it. Granted, it is sometimes extremely difficult to pinpoint the cause of the error, but if you are able to, you should provide that kind of information.

For example, if you are signing up for a new account, you may be asked to provide a password. Which of these three error messages would be the most helpful for you?

1. `Sorry, the request was unsuccessful.`
2. `Email address is improperly formatted or contains invalid characters.`
3. `The password has an incorrect number of characters. Please provide a password between 6-16 characters using any combination`

```
of upper-case letters, lower-case letters, and numbers.
```

The third choice offers a suggestion to the user as to how to fix the error they have created.

For the wiki, you may end up using JavaScript to accomplish some aspect of your design (JavaScript will not be covered in the scope of this chapter, however, so go out there and take a look at various HTML tutorials). While less common now, there will be a few users who have disabled JavaScript on their browser. What I like to do is to show a box by default stating "JavaScript should be enabled for an optimal browsing experience" and then using JavaScript to hide it when the page loads. This way, the box is hidden when JavaScript is enabled and is shown by default when it is not. This may be something that you can consider (though again, based on the popularity of many JavaScript-dependent sites, this may be a non-issue).

- **Help and documentation—***Even though it is better if the system can be used without documentation, it may be necessary to provide help and documentation. Any such information should be easy to search, focused on the user's task, list concrete steps to be carried out, and not be too large.*

  Supplement your interface with information, even simple instructions such as "click here to see what our project is about". This way, users are reassured that what they are about to do will accomplish what they want.

  iGEM wikis sometimes have a "tour" feature, where they lead the user through the various components highlights of the project without having the user look for the sections themselves. This may be something for you to consider as you build your own, but remember that a lot of other wikis do not do this.

9. HTML and CSS

HTML stands for *Hypertext Markup Language*, and is the markup language which all internet browsers understand. Plain, pure HTML only specifies **what content appears**, including text, images, links, tables, and divisions (known as *divs*). HTML by itself does not specify **how the content looks**—that is what CSS is for. CSS stands for *Cascading Style Sheets* and is a separate language (with its own separate syntax) that manipulates the positioning, colour, font, line spacing, and all visual aspects of an HTML document.

In a nutshell, internet browsers work by downloading an HTML document (and all the supplementary data such as images, CSS, and JavaScript) from the internet, interpreting it, and then graphically rendering it for the user to read. The iGEM wiki server is no different—the HTML is there, but it is wrapped in a layer of wiki markup. When you edit in wiki markup, the server translates the markup into HTML before sending it to the user's browser (the *client*). The person editing the wiki doesn't need to worry about scary-looking angle brackets and instead can focus on the content.

Editing the iGEM wiki in HTML is not as difficult as it may first appear. It is a matter of understanding what each individual component of the language does and how it affects what is displayed on the browser. Web development is a lot of trial-and-error in that respect.

10. But Wait, You're Not Teaching Me HTML?

There are many resources online for learning HTML and CSS, and they do a much better job than any attempt I can make to teach you the actual syntax of the languages. For this reason, this chapter will not be teaching you anything about the HTML and CSS syntax. **Instead** (before you scoff and throw

the Guidebook away in anger and disappointment), I am going to focus on how to utilize HTML and CSS in the iGEM wikis, since the server behaves differently from a traditional blank web page. Almost all tutorials that you will encounter online assume you are building a website on your own domain with minimal clutter. The iGEM server space provided is a shell that surrounds your wiki and therefore adds code that makes it a little harder to manipulate the page into something you want.

Before we go into that, I recommend that you take a look at the following tutorials that guide you through aspects of the languages step-by-step. These tutorials should not take you any longer than a few days of practice before you at least grasp the vocabulary and understand what the syntax looks like, as the final section ("HTML and the iGEM Wiki") will become rather technical:

> http://www.codecademy.com/tracks/web
>
> **Other reference material:**
> http://www.htmldog.com/guides/css/beginner/
> http://html.net/tutorials/html/
> http://www.csstutorial.net/
>
> **Required reading after finishing a CSS tutorial:**
> http://css-tricks.com/the-css-box-model/

Codecademy is an interactive tutorial course that teaches you the basics of HTML and CSS. It is incredibly valuable as a learning tool, so I recommend you start off on it first. Remember that Google is your friend. In this day and age of web design, there are many sites that can provide tutorials and solutions for almost any problem you encounter as you are building your wiki.

11. HTML and the iGEM Wiki

This section will assume that you understand vocabulary such as HTML *tags*, *elements*, *attributes*, *divs*, *ids*, *classes*, and the CSS *Box Model*, *selectors*, and *properties*. In this section, I will discuss some of the tricks that I have discovered as I continued developing wikis for the past few years. Unfortunately, a few of these workarounds are not going to be considered good web design practices but are sufficient to complete what you need to complete. If you do find solutions that are more effective than the ones I have described, congratulations (and please let me know about them)!

Tools Required

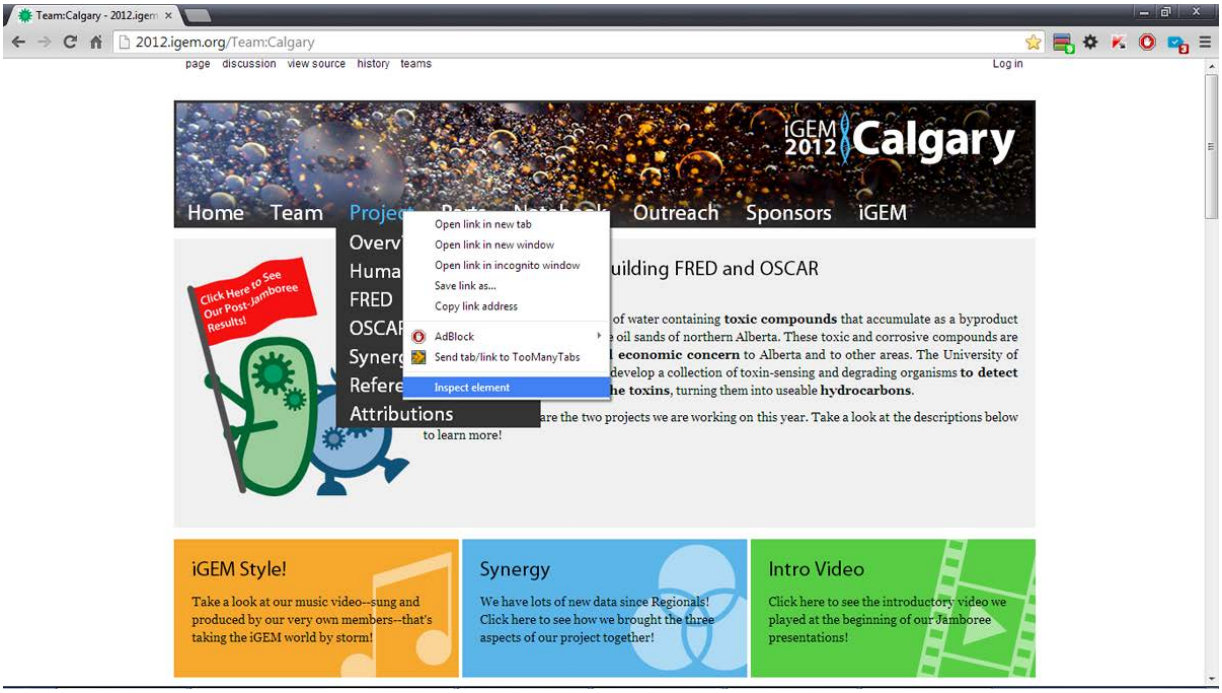
The major tools used in wiki building are a text editor and some kind of image editing software (Adobe Photoshop or Illustrator is a common standard, but if you are short on cash you can always find open-source software such as GIMP and Inkscape). For text editing, I recommend that you get an editor such as TextWrangler (for Macs) or Notepad++ (for Windows) as they can perform *syntax highlighting*—essentially, colouring in tags and keywords in your code to make reading easier. From here, you can copy and paste code you write onto the iGEM wiki.

The Element Inspector

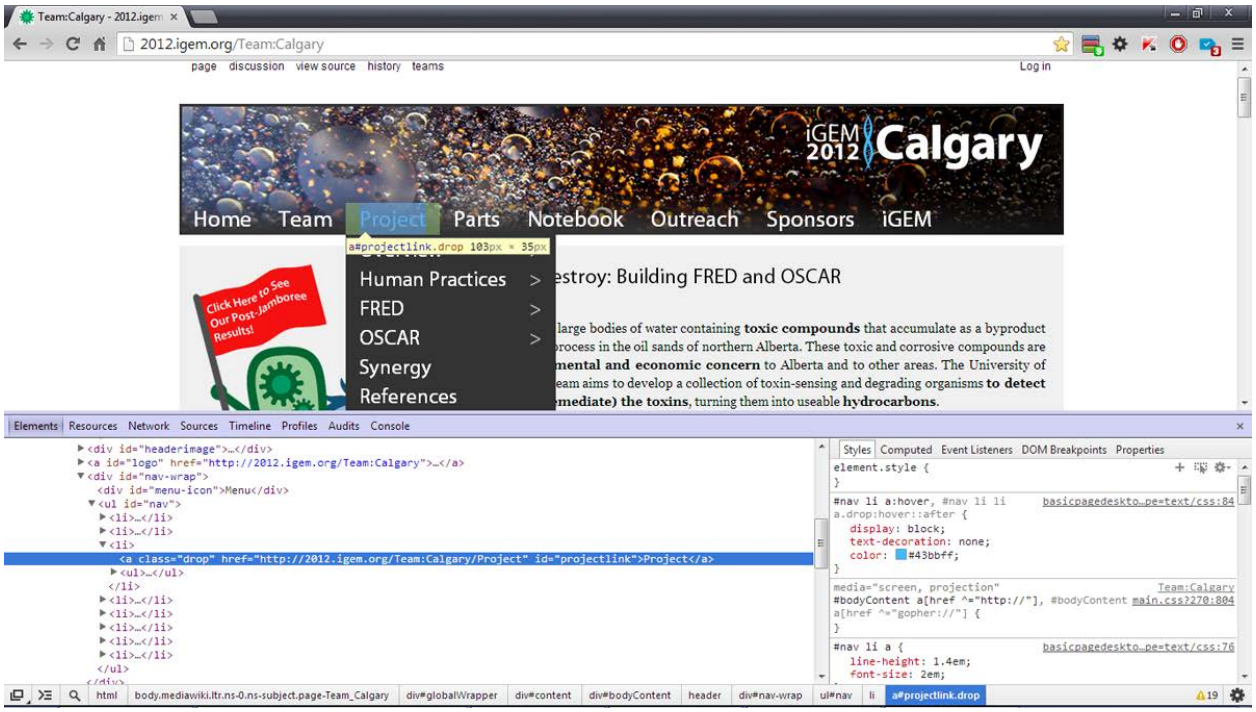
Your finest weapon during this process will be the ability to right-click on a webpage element and seeing exactly what part of the HTML and CSS creates it. You are able to "Inspect Element" on both Chrome and Firefox (and potentially Safari). Internet Explorer is capable of doing this, but only for the newest versions in Desktop mode (not Metro mode).

One of the benefits of the Inspect Element tool is to edit the CSS on the page itself to see how it can impact the layout of the page (temporarily—the changes are erased on refresh). For Firefox, you will require a plugin called Firebug, but it is native on Chrome.

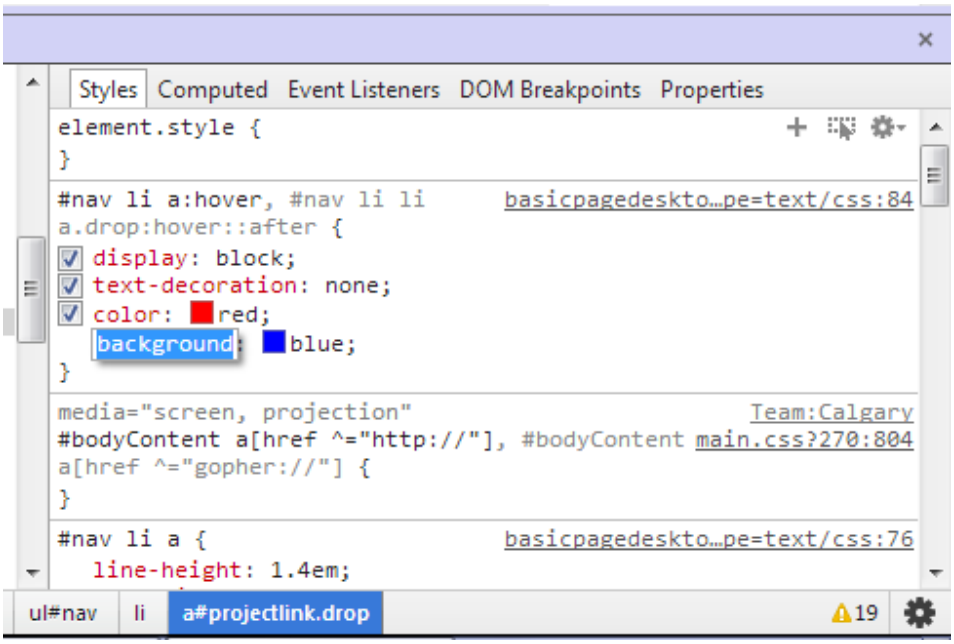
Therefore, if you find an element on a wiki that you are curious about, you can right-click it, use "Inspect Element", and play with the CSS rules displayed to see how it is affected, or create new CSS rules altogether.



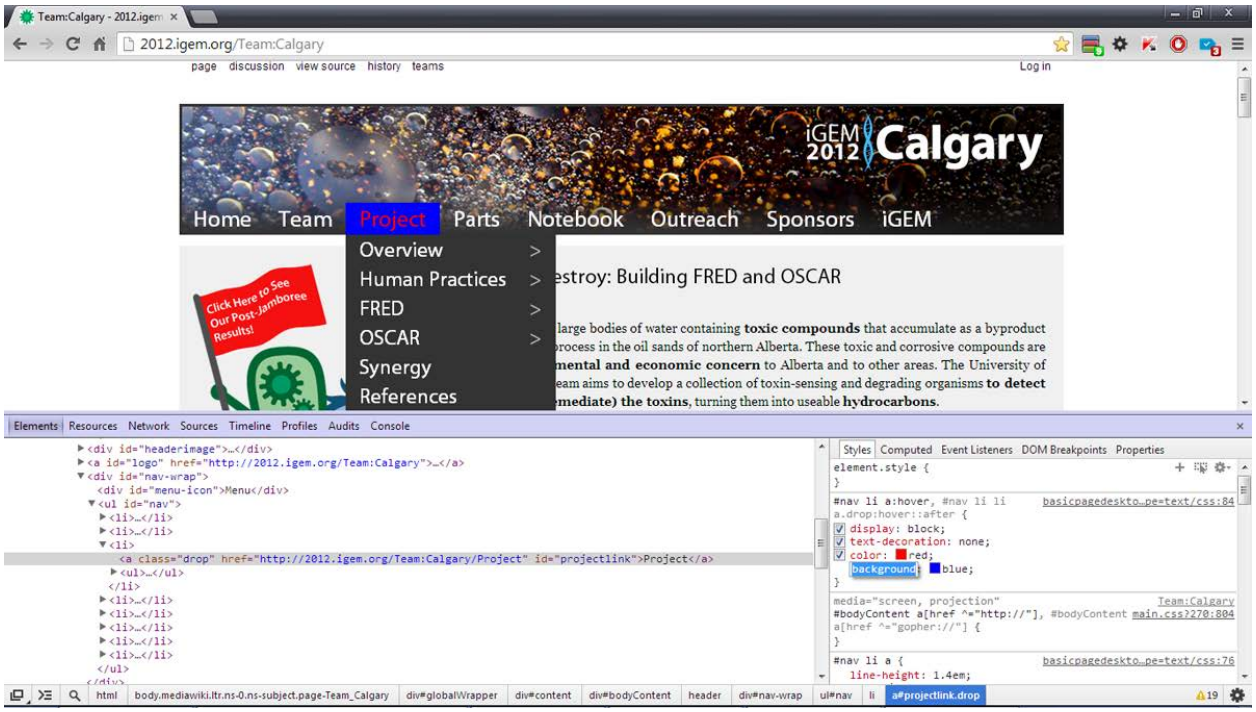
Right-clicking on an element and clicking "Inspect Element" allows you to examine it in further detail.



Mousing over particular lines on the HTML side shows you the element's location, as well as parts of the Box Model such as padding, margins, and borders.



Clicking on an empty area in the CSS area creates a new CSS rule. You can also change existing properties and values of the element. Pay attention to the selectors. If you want a rule for just the element you are highlighting, use the `element.style{}` selector at the top instead.



You can see that by changing font colour to red and background to blue, this particular element has now adopted new temporary CSS properties. These changes are erased when the page is reloaded.

Switching Between HTML and Wiki Markup

In order for your browser to understand your HTML code, you can simply put everything in the iGEM wiki editor between two `<html>` tags. CSS and JavaScript go between `<head>` tags, and the rest of the content goes into `<body>` tags. Rather straightforward and, in general, not an issue.

However, if you are using snippets of wiki markup (such as when using templates), you have to close the `<html>` tag you are working with, enter the wiki markup, and then reopen the `<html>` tag again. This tells the browser to stop interpreting in HTML and begin to interpret in wiki markup.
For example, the following snippet adds a thumbnail image in a wiki image box:

```
<html>
<body>

<p>Paragraph of text.</p>

</html>[[Image:test.png|400px|thumb|caption]]<html>

</body>
</html>
```

Templates

One of the nice aspects of the iGEM wiki is the ability to utilize wiki markup to create templates. Templates can hold stylistic elements, CSS, and JavaScript which can be reused for all pages of a wiki. The benefit is that if there are changes to be made to the template, such as adding a new item to a

navigation menu, it will automatically apply to every page that utilizes that template, without requiring you to find and test every link.

Templates are the exception to the rule that you must have all wiki pages within your own wiki namespace. Templates do not work if you create one under your own namespace. They must come directly from the main iGEM site.

http://2014hs.igem.org/Template:TemplateName

Note how there is no indication of whose team this template belongs to. Theoretically you could use any team's template from that year (but why would you?) If you want to be very specific about whose team the template belongs to, you are able to use colons and spaces after the *Template:* keyword.

http://2014hs.igem.org/Template:Team:Example_Canada/TemplateName

Template pages are created and edited like any other wiki page, but it comes with an extra feature. In wiki markup, if you use triple curly braces, `{{{ }}}`, you can create *parameters* in the template. When the template is used in your wiki, you can then put content into those parameters without affecting the template's CSS and other elements. Therefore, you can hide the HTML and CSS of things such as sidebars, fonts, and stylesheets in your main wiki and make it easier for the rest of your team to edit. (They will thank you for it. And you will thank them since there will be less risk of them accidentally editing important structural elements on the page.)

Further details on how to use parameters in templates can be found here: http://meta.wikimedia.org/wiki/Help:Template

Remember that since templates are **not HTML**, but rather are specific to the wiki itself, it will not be recognized by the browser if parameters or other features are within `<html>` tags.

In order to use a template on a normal page, you use two curly braces and then specify the name of the template without the word *Template:* at the beginning. Parameter values can then be entered, separated by pipe characters, |, and then closed with two curly braces.

For example, if you were using a template with the location *http://2014hs.igem.org/Template:Team:Example_Canada/MainTemplate* with the following:

```
<html>
	<body>
		<p>This is text that appears on all pages.</p>
		</html>{{{TEXT|This is placeholder text}}}<html>
	</body>
</html>
```

You can then use it on any other page like so:

```
{{Team:Example_Canada/MainTemplate|
TEXT=
	<html>
		<p>This overrides the placeholder text.</p>
	</html>
}}
```

Note how you need to have the `<html>` tags again when you're filling in replacement HTML content. Without them, the browser would interpret the `<p>` tags inside as wiki markup and show them. Also

note how there is no *Template:* present when specifying the template—this is already implied when you utilize the double curly braces.
The link provided will have more examples on how to properly use a template. If a template is successfully used, you will see it listed on the bottom of the editing window of the normal page when you edit it again. If you like, you can start modularly stacking templates together—templates can use other templates and pass on parameters by making it their own! Take a look at some of the collegiate wikis of years past to see how they used their templates.

External Stylesheets and JavaScript

To minimize errors in redundancy and to save the amount of data that a browser needs to download, many sites will have their CSS and JavaScript externally, in a separate file from the HTML document. When a browser downloads an HTML document for the first time, it downloads a copy of the CSS and JavaScript (if it is external) and stores it locally. This speeds up the download of all other pages that use the same CSS. If CSS rules are found on every page, the browser has no choice but to download it each time.

Therefore, it is good practice to have your CSS stylesheets uploaded separately on the iGEM wiki and referenced within your own CSS as an external file.

In order to do this, you simply create a new wiki page and paste the CSS into it, with no extra HTML tags. However, you will discover that the page will look rather strange, as it interprets the CSS as wiki markup and formats it accordingly.

Take this CSS sheet for example:
http://2013.igem.org/Team:Calgary_Entrepreneurial/Stylesheets/global.css. If you click "View source" or "Edit" in the top menu, you will see that it is in fact properly formatted CSS. The wiki interprets it otherwise, however, and if you insert this link directly into your HTML the page will not be rendered correctly.

The solution is to add the snippet `?action=raw&ctype=text/css` to the end of the page's web address. This forces the wiki server to show the file as raw CSS. Observe the difference between the link above and this one:
http://2013.igem.org/Team:Calgary_Entrepreneurial/Stylesheets/global.css?action=raw&ctype=text/css

The second link can be properly interpreted when you use it in a `<link>` tag on your pages:

```
      <link rel="stylesheet"
href="http://2013.igem.org/Team:Calgary_Entrepreneurial/Stylesheets/glo
bal.css?action=raw&ctype=text/css" type="text/css"/>
```

As for JavaScript, I have not been able to determine what the `ctype` can be changed to that lets the browser interpret it as external JavaScript. For my own purposes, since JavaScript is used lightly in the wikis I have built, they remained inline in the templates that I utilized. If there is a snippet that can be used for external JavaScript, by all means use it.

External Stylesheets Using Templates

If the above method of external stylesheets doesn't suit your fancy, you can also create a template, plug in all of your CSS code (between `<html>` and `<head>` tags) and save it. If you've done it correctly, you should see no content on the template page (since everything is in CSS only). Then you can utilize this CSS template in your pages like any other template. It is a simpler method of adding CSS to your pages, but since it is generated each time the page loads, it may not be cached in the browser like the above method does.

Images

Remember that there are three links that you can use to find an image file you have uploaded. For `<img>` tags, you **must** use the direct, full-resolution link in the `src` attribute. The other two do not work since they are wiki pages with their own markup. If you are not using `<img>` tags and are instead using wiki markup to put up figures, you must use the wiki server filename (with all spaces replaced by underscores, _).

Responsive Web Design and Media Queries

You may see a lot of articles online talking about responsive web design. This may not be of any concern to you (there is, in fact, a lot of extra work involved in making a website truly responsive), but if you are brave and committed, one of the easiest ways to create responsiveness is through media queries. However, while a lot of sites will say that you should have the media queries within the `<link>` tags and have multiple external CSS files, it does not work on the wiki. Instead, you must have **all media queries within a single CSS** file that is then linked externally.

An example can be found on the 2013 Calgary Entrepreneurial wiki's CSS: http://2013.igem.org/Team:Calgary_Entrepreneurial/Stylesheets/main.css?action=raw&ctype=text/css. Note how the media queries are present in this one file.

The `!important` Statement

CSS is known as "cascading" because rules written later in the document override rules earlier in the document. This allows very general CSS rules to be established at the beginning of the document and very specific rules later on in the document.

However, sometimes a CSS rule doesn't stick no matter what you try. This may be due to the presence of `!important`, a rule appended to the end of a CSS statement that causes it to override all other rules regardless of where it is in the document. The iGEM wiki may have `!important` statements in its own CSS that overrides a rule you are trying to implement. `!important` statements cascade with other `!important` statements, so this might help.

Use this technique sparingly. It can cause you severe headaches down the road if you abuse this feature because the logic behind how CSS rules interact with each other is broken.

12. Conclusion

Hopefully this chapter has given you some groundwork on beginning to build your own wiki. Remember to keep the end user in mind as you continue building your wiki. Look for examples of Best Wikis in both the High School and Collegiate divisions for inspiration on how to further improve your own.

HTML and CSS are rather tricky to learn, but you will begin to grasp the behaviour of the code as your continue to practice. Don't be afraid to experiment and don't be discouraged if something doesn't seem to work quite right. The internet is a great resource—it's very likely that if you are having a problem, a lot of other web developers have had similar ones. You can always find tutorials online that teach you how to build specific website features, like a fixed sidebar or a dropdown navigation menu. Tutorials are an excellent way to see how HTML, CSS, and JavaScript can work together in order to build something tangible.

If this chapter has sparked some interest in UX and web design/development, there are literally millions of UX resources online that you can Google. Keep in mind that since UX is rapidly changing

with rapidly changing technology, there are lots of heated debates about whether or not specific user interface elements are good UX in this day and age (dropdown menus, for example, remain a big point of contention because of issues regarding touchscreens.) The tips and tricks shown here, and even the Nielsen heuristics, are all rules of thumb that can be broken if it ultimately improves usability.

Remember that UX is about **providing a good experience** for those who visit your wiki, and if your users are able to use your interface and design effectively with minimal frustration, the design has not failed. Don't feel that these rules are restricting your creativity. Science and art are both about experimentation, and taking in those results to further improve.

With that, I wish you all the best of luck in your projects, and I look forward to seeing how your wikis turn out!

11. POSTER AND PRESENTATION

Lisa Oberding and Zak Stinson

1. Introduction

At the Jamboree, you will be required to present your iGEM work by giving a 20 minute presentation, as well as presenting a poster explaining your project. These presentations, along with your **Wiki**, are how your project is judged. The presentation and poster components of your project are very important, as you must be able to explain to others what research you performed over the summer, why you did it, how you went about doing it, and what you learned. In scientific research, presentations and posters of research projects are presented in order to teach others about new research methods and discoveries. Research must be published and presented to others in order to allow others to learn from it and build upon it, furthering the collective knowledge of how things work. Even the most fascinating research discoveries or valuable new methods of doing things are basically useless if they are not presented so that others can learn from them.

Past posters, presentation slides, and video of team presentations can be found at http://igem.org/Results. The default division is the collegiate division, but high school teams can be viewed by clicking "High School". By clicking a year and region, a list of teams will be brought up, showing the team name as well as any prizes won. Next to the team name there will be icon links to the teams' poster, presentation slides, as well as a video of their presentation during the jamboree. It is useful to look through past presentations and posters for both high school teams and collegiate teams, as well as watch videos of past team presentations in order to get an idea of how these components are structured and executed. It is useful to look especially at teams who have advanced to the finals (in the collegiate division), who have won a special award for presentation or poster, or a title at the jamboree. Use these past project components as inspiration, but be careful not to plagiarise and copy them directly.

2. The Presentation

During the Jamboree, each team will give a 20 minute presentation to both the judges and other iGEM participants. Each team is given 20 minutes of presentation time, 5 minutes for questions and answers, and then there is 5 minutes to switch with the next presenters. Please be sure to bring all the necessary equipment for your presentation, such as your laptop, cables/adaptors, and power supply, as iGEM will not be providing it. To be able to use PowerPoint slides during your presentation, you must have a PDF version of your PowerPoint file and your poster on a flash drive, ready to give to the iGEM staff before you present.

The purpose of the presentation is to explain your project to the audience in a way that will allow them to understand what you have done, why you have done it, what was involved, and what results you obtained. The presentation must be given by student members of your team using PowerPoint presentation projected on a screen behind the presenters, which enforces you to know the contents of the presentation prior to the day of the presentation. The 20-minute time limit is strictly enforced and speakers will be cut off if this limit is passed. Although there is no required format for your presentation, there are certain guidelines to keep in mind in order to have as effective a presentation as possible.

Components

1. Title Slide: It can be confusing to the audience if you dive right into the story of your project without addressing the audience first. Your presenters should briefly introduce themselves and let the audience know what team they are from and the name of the project. A creative name helps to make your project stand out, as does a catchphrase that gives the general idea of your work in a clever way. Ideas for titles and catchphrases can be found by browsing through past projects. Having a memorable name, catchphrase, and/or logo can help judges to remember you and gets the audience interested. Your title slide should have thought put into it, even if it is just your team or project name. Remember, this is the first slide that the judges and the audience will see of your presentation, and it will set the tone for what they are about to watch.

2. Background: Your project will likely be super interesting to you and your team, because it is what you have dedicated all of your time and energy to in the past few months. Your team will be very familiar with it and know quite well why it is interesting and useful. However, it is important to remember that the other teams and the judges aren't as familiar with your project. It is your job to get them as excited about your accomplishments as you are. To do this, you must get the audience up to speed using some kind of a background to your story before you launch into all of the things you did. Otherwise, you will lose your audience and nobody will have any clue what you are talking about or why they should care. You must explain clearly what it is you are doing and –very importantly- why you chose to do this. Your team must have picked this particular idea or project for a reason - what was it? What was interesting about it? What was the problem you were trying to solve or the goal you were trying to achieve? Why was it important? Always remember that you are presenting to people from different fields of study from all around the world - it will be important to explain what you did and why it is important in a clear, accessible, and captivating manner. The introduction is one of the most crucial, if not the most crucial, parts of your presentation as it is the beginning of your story. This section sets the tone for the rest of your presentation, and determines whether the audience is interested and will understand your project.

3. Your Project: Once you have explained the problem you were trying to solve and why you chose that problem, you can begin talking about how you solved it - your project work. This is where you explain your experiments, results, and everything else that you did for your project over the summer. From here on in, the layout of your presentation is far more flexible, and how you choose to explain your project will depend entirely on what your project is. Some projects may have sub-project components and are most easily explained in sections, with a final conclusion as to how everything ties together. Other projects have only a single component, and it makes more sense to explain each step and how it led to the final result. Since you will spend the longest on this section of your presentation, it is useful to consider the following tips:

    a. It is not necessary to discuss every little experiment you did and every method you used in your work. Rushing through explanations of every method to show all of your data will be stressful to your presenters and make it hard for the judges to follow. You should aim to discuss what you did and how you did it in general terms, and only highlight the most important experiments, i.e. those that gave you a results that were significant in showing your project working. Keep in mind that judges will always have access to your project's Wiki where you have documented every experiment. During the presentation, you want to highlight the most exciting or interesting experiments that help to show you accomplished what you set out to do with your project.

    b. Experimental results on constructs that were assembled and tested by your team must be highlighted. These results have come from your hard work, as you had to both build and test these constructs. Make sure the judges know that these were constructs that you built!

c. Explain in general terms how you did your experiments, but don't give exact protocols - your audience does not need to know all the details. Explain only what the audience needs to know in order to understand what you did. Your audience will likely be familiar with general procedures like restriction digests and cloning, so it will not be necessary to go into procedural details in your presentation unless you have done something unusual.

d. Make sure to explain clearly the meaning of the experimental results you present and why they are important to your project. For example, instead of saying "there was a band on the gel", say "the band on the gel when we digested our plasmid was the expected size of our gene, meaning that our part contained the gene we were looking for". Or, instead of only saying "the cells turned green", say "the cells turned green when we transformed them, indicating that they must have our **DNA** part in them and the DNA construct is functional and expresses the green fluorescent protein".

e. Show your data in a way that gets the point across clearly and quickly: Your audience will likely be seeing a slide very briefly. Using a color coded graph with a clear legend, or some good pictures of your cells will be much more effective in getting your message across quickly and clearly than a table filled with numbers. You can also make sure you have a schematic for your audience to follow along which points precisely to the part of the project you are referring to.

4. Conclusion: Here, you want to bring everything together. What was the final system or construct you came up with and how did you show it worked? How do the components or subprojects come together? Whether at the time of the presentation you have a full system or only the components completed, go back to the big picture of what you said you were trying to accomplish and explain what you accomplished so far. Explain the implications of what you created and how it could be used - both in relation to your chosen problem and to other possible future applications. How is the outcome of your project helping to solve the problem that you chose? Which aspects of your project could be pursued next? This is your last chance to convince the audience, in your own words, about what you accomplished and that your project worked. Make sure you convince them.

5. Attributions: iGEM teams rely heavily on help from sponsors as well as various other people. At the conclusion of your presentation, it is important to thank all of them for their help. If you forget to do this, you can not only lose points but also jeopardize future relationships with sponsors and other support networks. Generally, this is done using a slide or two with the names of everyone who helped you and the logos of your sponsors. You don't have to read every single name out loud - Just a general thank you will do as long as you acknowledge everyone. Following this, you can wrap up your presentation by letting the audience know you will take any questions or comments they may have and thanking them for their attention.

6. Q&A: During the question session, you will be asked questions not only on what you just presented but also on what is on your Wiki. Be prepared in advance for what might be asked. The best preparation for this is to practice, practice, and practice!! Have mock question sessions with your team and advisors beforehand in order to test your knowledge, and get everyone to read the team Wiki. Not everyone needs to have 100% knowledge of every detail, but everyone on the team should know the general details of what was done in **every** aspect of the project, and the **specifics** of the section that they were most involved in. Here, It is a good idea to designate a team member or advisor to direct questions to specific students to keep things organized. It is also good to have only one team member answer a particular question, and limit others jumping in unless the first team member missed an important point.

Tips

1. Avoid, at all costs, a wall of text. Your slides should have figures and pictures, with key bullet points to convey the main ideas of that slide - do not write full paragraphs on a PowerPoint slide! The slides are there to highlight what you are saying, show figures and graphs, show key images, and emphasize important points in the words you are saying. Your slides should NOT be used as a script you are reading directly off of. Having too many words on a slide is distracting and unpleasant for the audience as they will be trying to read quickly instead of listening to you.

2. Make sure the fonts you use are readable. They need to be large enough that people sitting in the back row can read them (25pt or more for fonts like Arial). Also, while decorative fonts might seem nice, they are very hard to read on a screen from far away. Keep the fonts simple, just like you would for your Wiki. In fact, following your wiki design guidelines for your PowerPoint will help you make an awesome slide deck to go with your oral presentation.

3. Figures should be clear, crisp, and easy to see - not pixelated. They should also be as simple as possible: if you have to use complex figures, make sure to highlight and point out the part that the audience should care about. When presenting data in a figure, label it clearly and make sure your labels are big enough to be seen by your audience. Tables should be kept simple, if used at all. Explain your data figures and tables during your presentation. What are they supposed to show? Don't just put them up and expect your audience to understand the significance of the data without any further explanation.

4. Pictures are good, but in moderation. Don't cover your slides in random images - it is very distracting and looks messy. Pick pictures which convey your point best. If you must have a lot of images try to separate them onto separate slides.

5. Color schemes: You want your presentation to be readable, as well as visually attractive. Don't insert a crazy background - although it might look fancy, if it makes it harder for your audience to read, it will do more harm than good. Stick to simple color schemes that are not super bright.

6. Animation - keep it minimal. Having text appear on a slide is fine - provided that it is well rehearsed and it helps the audience follow the presenter, who can click to bring up the next point for discussion. DON'T have it spin and twirl and get bigger before coming in - remember you want something that is simple and easy for your audience to read.

7. Have titles on your slides - These should be short and serve as reminders of what you are talking about. When you are moving to new section of your presentation - say, from "experimental design" to "experimental results", or from one subsection to another - use a transition title slide where you can explain that you are now moving into this new section ("now that I have shown you X, I will talk about Y").

8. Your presentation is more than just a PowerPoint! The oral presentation is what grabs the audience's attention and tells a story. The PowerPoint is there to back up the presenters. Try not to read directly off of your slides. Instead, use the key points on the slides to remind you of what you need to say. Rehearse the presentation multiple times - as many times as you can - and request feedback after each round. When presenting, try to make eye contact with the audience, smile, move around a little bit (but try not to fidget). Not only will this look good to your audience, but it will help with your nerves as well. Remember- <u>you know what you are talking about better than anyone else in the world when it comes to talking about your own project.</u> The audience is not there to tear you apart - they are actually very interested to hear what you have done and what you have to say. Just talk to them like you would a classmate! If you are very nervous, look for your team in the audience and some other smiling faces, and

focus on presenting to them with a few quick glances at the judges. And if you feel like you are stuck on saying something- take a breath and just move on to the next point. Don't try to struggle with what you thought you needed to say.

9. It's usually easiest to have a few presenters only; at most 4. When trying to hand the microphone off to every member of the team, it can get hectic. However, you are free to do so, and many teams do choose to present together. Go with whatever works best for your team and your story.

10. **MOST IMPORTANTLY: Practice, Practice, Practice.** Start in advance. Present to your friends, your family, your teachers; anyone who will listen! Start working on your presentation as soon as possible. The more time you have to work on the presentation, the better it will get, and the easier presenting it will be. Presenting material to people other than your teammates who know the project well will ensure that you are communicating your ideas in an interesting and effective manner. Can you explain your project to your mom or dad who is educated individuals but not necessarily experts in your field of study? If the answer is yes! Then you have done your job effectively.

3. The Poster

Each High School team is required to present a poster at the Jamboree. Inside the team package you will receive at the Jamboree, you will find a list of locations assigned for team posters. A timeline along with guidelines for the poster sessions will be available- posters must be put up and presented during specific times. The poster must be no larger than 48 in x 48 in (1.22m x 1.22m, 4ft by 4ft). However the maximum size of a poster is very large and it may be advantageous to make a slightly smaller poster if possible. Keep in mind that you will need some type of tubing to protect and transport your poster to and from the competition- you can find things that will work at art stores and hardware stores. Before your presentation, in addition to submitting a PDF file of your Power Point presentation, you will need to submit a PDF file of your poster as well.

All teams will hang their posters up in a designated area, and will present their posters during a poster presentation session scheduled in advance. The session is usually scheduled for two hours. During this time the judges, as well as members from other teams, will circulate to examine the teams' posters and ask questions to team members about their projects. Two types of judges will examine your poster; (1) judges that specialize in marking scientific posters, and (2) judges who also evaluate presentations and Wiki's. Some judges will be more specialized in a particular area of your project than others.

Each student on the team should be able to explain the project using the poster - the goal is to explain the story of your project to anyone who asks in a short 5-10 minute talk, using the poster to highlight the most important components. During the poster session you may be asked questions about any component of the project, so it is important to be prepared. Try to have at least one team member "specialist" for each specific component (such as **human practices** or modelling) of your project near the poster at all times in case a judge interested in that area swings by- this way team members who don't know specific details can pass the judge off to someone who knows that component in depth.

Components

In general, the layout of a scientific poster will have the same components as the project presentation:

1. Title: This should be large enough to see from far away, and possibly have some kind of hook or phrase with it in order to draw people in closer to view the poster.

2. Authors: This is where the names of your team members and advisors are listed. Generally this is in alphabetical order by last name, with student members before advisors. The format is usually "Last Name, First Initial., Last Name, First Initial."

3. Introduction: Like the oral presentation, your poster's introduction should tell the story of what you are trying to do, the background to your problem, and why it is important.

4. Project: The components of the project can be split up differently depending on the structure of the project. Some teams create separate sections for each sub-project, containing methods and results for each. Some will have separate sections for methods and results. Some projects use their Human Practices component as their introduction, while others have a separate section for this component on the poster. How your poster is laid out will depend on your project, and the best way to figure out how to lay out a poster is to look at other posters - see other teams' examples in the Results Wiki as mentioned in the introduction to this section.

5. Conclusion: Like the oral presentation, this section should wrap up your project, showing how the components come together, what the further applications of the project could be, and a summary of the results obtained when testing the final system. Again, this section will depend very much on the structure of your project, and viewing other posters for examples will be helpful.

6. Attributions: There should be a section on your poster for attributions and sponsor logos - Remember, points can be lost if this is not present.

Tips

1. Design guidelines are similar to those for the presentation and Wiki: Keep colors simple, make sure the font is large and easy to read (generally ~19pt minimum, no fancy swirly fonts, no dark on a very light background or vice versa), and keep a good balance of text to pictures: you should only use short sentences at maximum, bullet points would be best, with images of results and concepts if possible.

2. Different sections on the poster should be clearly separated in order to make the poster easy to read. This can be done by using boxes or borders of some kind, as well as different colored backgrounds. There should also be a clear title for each section.

3. Sections should be ordered logically - Keep in mind that people will either read left to right, or top to bottom. Place your sections in order in rows or columns accordingly to make the flow of your project story easy to follow.

4. The content shown on your poster should highlight only the most important results. You have very limited space, which means you want to present the story of your project in a concise yet captivating fashion. Make sure all the important points are touched upon.

5. Figures should match the color scheme of the poster and be as simplified as possible. They must also be clearly labeled (axis, legends, etc.) and have a concise figure caption (above tables, below figures). Figures should be numbered in the order in which they appear on the poster from where the audience would start reading to where they would end (note: sponsor logos don't get a number).

6. There should be around 3 students at your poster at all times during the poster session, but not more! You don't want to crowd the poster area with your team. Have the rest of the team members go check out the other posters and trade off if necessary. It is best to have team members with detailed knowledge of each section close to the poster at all times, so that questions can be directed accordingly if someone wants more detail about a particular aspect.

However all your team members (including those NOT near your poster) can be asked questions and should be able to tell the story of the project. Again, practice as much as possible.

7. Ask people approaching the poster what it is they would like to hear: do they have questions, or do they want to hear the overall story of the project? This way, you can direct what you say accordingly.

8. Be active in approaching people. If someone is lingering near your poster, approach them. Have other team members mingle throughout the area; some teams create flyers or have other things like key chains to hand out and draw people in to their posters. You want to get as many people as you can to come see your poster and stand out in the crowd as much as possible (bright shirts, costumes, flyers) in order to get the judges to remember you and to come see your poster.

9. Remember you are telling a good story - you want to explain your project like you would in the presentation; what you are trying to do, why it is important, how you did it, and what results you obtained.

10. Have fun. Meet people, ask them questions about their projects, make new friends - Poster sessions can be stressful, but they are also a great opportunity to meet other people from all around the world and make new friends.

Appendix 1.

PROTOCOLS

Magdalena Pop and Himika Dastidar

ASSEMBLY OF PARTS

1. Using DNA distribution kit plates: Rehydration of registry DNA from kit plate

Wells in the DNA distribution kit plates contain 2 - 3 ng of dry DNA. This should be enough DNA for a few transformations, but insufficient for immediate restriction and ligation.

Materials

DNA distribution kit plates	micropipette
sterile distilled water	sterile micropipette tips

Protocol

- Locate the well with the DNA part you need.

- Using a micropipette, draw 10 µL sterile distilled water into a sterile pipette tip.

- Use the pipette tip to puncture a hole through the cover foil and into the well containing the DNA you need.

- Release the water into the well and pipette up and down a few times to rehydrate the DNA. Wait for about 10 minutes to ensure that all DNA has been re-hydrated.

- Store the re-hydrated DNA in the - 20 °C freezer. You can either leave it in the original plate, or transfer it into a separate tube (make sure to label the tube!).

- The re-hydrated DNA can be used immediately to transform competent cells. As little as 1 µL should be enough for one transformation.

2. Restriction digestion

This protocol is for cutting approximately 500 ng of plasmid DNA with two different restriction enzymes. This should provide enough cut DNA for both ligation and agarose gel electrophoresis.

Materials

plasmid DNA	(0.6 mL) microcentrifuge tube
10x restriction buffer	microcentrifuge (optional)
sterile distilled water	ice
restriction enzymes (EcoRI, SpeI, XbaI, and PstI)	micropipettes and sterile pipette tips
thermocycler	water bath at at 37 °C (optional)

Protocol

- Thaw all solutions and buffers needed. Homogenize the contents by gently flicking the tube. If possible, collect all liquid at bottom of tube by spinning briefly in the microcentrifuge at maximum speed. Place tubes on ice. RESTRICTION ENZYMES SHOULDN'T NEED THAWING AND SHOULD BE KEPT ON ICE AT ALL TIMES!

- Label a sterile 0.6 mL microcentrifuge tube with an identifying label for the particular restriction digestion you're doing. Make sure that you use a permanent marker and the tube fits inside the heating block of your thermocycler.

- Pipette the following into the tube *in the order listed*. It adds up to a total volume of 20 µL (see Note 1)

7 µL sterile distilled water

10 µL plasmid DNA (see Note 2)

2 µL 10x restriction buffer (see Note 3)

0.5 µL Restriction Enzyme #1 (see Note 4)

0.5 µL Restriction Enzyme #2 (see Note 4)

- Mix gently by pipetting up and down a few times. You can bring all the liquid to the bottom of the tube by spinning it briefly in the microcentrifuge at maximum speed.

- Incubate at 37 °C in water bath or thermocycler for 30 minutes to 1 hour.

- Incubate at 80 °C for 20 minutes. This will denature the restriction enzymes, which otherwise may interfere with subsequent steps.

- Store the restriction digests in - 20 °C freezer, or proceed immediately with ligation and/or agarose gel electrophoresis.

Note 1 - The total volume can be adjusted according to needs and DNA concentration. For example, if you don't want to waste any of your DNA solution, you may want to set up a total volume of 10 µL. Or, if you have a more dilute solution of DNA, you may want to set up a larger total volume, for eg. 40 µL.

Note 2 - This assumes a DNA concentration of approximately 50 ng/µL (10 µL x 50 ng/µL = 500 ng total DNA). The DNA concentration can be measured with a spectrophotometer. If you have no access to a spectrophotometer, you can estimate the DNA concentration from the yield of your plasmid miniprep kit and by agarose gel electrophoresis.

Note 3 - Restriction buffers are usually 10 fold (10 x) more concentrated than needed in the final restriction digest. Therefore, they need to be diluted 10 times, i.e. 2 µL in a total volume of 20 µL, 4 µL in a total volume of 40 µL, etc. Also, make sure that both restriction enzymes you're using will cut under the buffer conditions you selected! For choosing the correct restriction buffer, check out this New England BioLabs (NEB) tool.

Note 4 - Restriction enzymes most commonly used in iGEM are EcoRI, SpeI, XbaI, and PstI. Selecting which two restriction enzymes to use will depend on the plasmid DNA to be cut and the particularities of your project.

3. Ligation

Materials

insert restriction digest mix	(0.6 mL) sterile microcentrifuge tube
vector restriction digest mix	microcentrifuge (optional)
sterile distilled water	ice
10x ligation buffer	micropipettes and sterile pipette tips
T4 DNA Ligase	thermocycler

Protocol

- Thaw all solutions and buffers needed. Homogenize the contents by gently flicking the tube. If possible, collect all liquid at bottom of tube by spinning briefly in the microcentrifuge at maximum speed. Place tubes on ice. THE LIGASE ENZYME DOESN'T NEED THAWING AND SHOULD BE KEPT ON ICE AT ALL TIMES!

- Label a sterile 0.6 mL microcentrifuge tube with an identifying label for the particular ligation reaction you're doing. Make sure that you use a permanent marker and the tube fits inside the heating block of your thermocycler.

- Pipette the following into the tube *in the order listed*. It adds up to a total volume of 10 µL (see Note 1)

3 µL sterile distilled water

3 µL of *insert* restriction digest (see Note 2)

2 µL of *vector* restriction digest (see Note 2)

1 µL 10x ligation buffer (see Note 3)

1 µL T4 DNA Ligase

- Mix gently by pipetting up and down a few times. You can bring all the liquid to the bottom of the tube by spinning it briefly in the microcentrifuge at maximum speed.

- Incubate at room temperature for about 1 hour.

- Incubate at 80 °C for 20 minutes. This will denature the ligase, which otherwise may interfere with transformation.

- Store the ligation mix in - 20 °C freezer, or proceed immediately with transformation.

Note 1 - The total volume can be adjusted according to needs and the concentration of DNA in the restriction digests. You should aim for an approximate *molar* ratio of 3 : 1 between the *insert* DNA (the segment of DNA that is inserted/added) and the *vector* DNA (the linearized plasmid backbone to which the insert is added). Also, the amount of DNA in the ligation should be low, i.e. around 100 ng total DNA.

Note 2 - It is assumed that the DNA concentration is the same for both *insert* and *vector* restriction digests, and it equals approximately 25 ng/µL. This would be the concentration if both restriction digests were set up according to the protocol above (see **Restriction digestion of plasmid DNA**). Assuming the insert is about half the size of the vector (e.g. insert = 1000 base pairs or 1 kbp and vector = 2000 base pairs or 2 kbp), to get a *molar* ratio of 3 insert : 1 vector in the ligation, their *volume* ratio must be 1.5 insert : 1 vector.

A general formula for determining the volume ratio of insert to vector (Vi / Vv) in the ligation mix is provided below:

Vi / Vv = 3 x (Cv / Ci) x (Mi / Mv)

where Cv = DNA conc. of *vector* restriction digest

Ci = DNA conc. of *insert* restriction digest

Mi = Molecular weight i.e. approximate size of insert

Mv = Molecular weight i.e. approximate size of vector

Note 3 - Ligation buffers are usually 10 fold (10 x) more concentrated than needed in the final ligation mix. Therefore, they need to be diluted 10 times, i.e. 1 µL in a total volume of 10 µL.

4. Transformation

Materials

100 µL aliquots of competent cells	ice
DNA ligation mix	micropipettes and sterile pipette tips

control plasmid DNA	water bath at 42 °C (or 37 °C)
SOC medium	incubator 37 °C
LB agar plates (with desired antibiotic)	spreader
	Bunsen burner

Protocol

This protocol is for one transformation. However, good practice demands that you always include controls. In this case, **a positive control** would involve transforming another portion of competent cells with a control plasmid DNA (if you get colonies you'll know that your competent cells and transformation protocol worked); **a negative control** would involve plating untransformed cells (you shouldn't get any colonies here - if you do, it's either because the antibiotic selection didn't work or there has been some contamination)

- Obtain microcentrifuge tube containing 100 µL competent cells. These can be either freshly prepared or taken out of the - 80 °C freezer. COMPETENT CELLS MUST BE THAWED AND KEPT ON ICE. Thawing on ice can take up to 10 minutes.

- Label the tube.

- Using a sterile pipette tip, add 2 - 5 µL ligation mix to the competent cells. Mix gently by flicking the tube. The rest of the ligation mix can be stored in - 20 °C freezer.

- Incubate on ice for 30 minutes.

- Incubate at 42 °C for 60 seconds. (Alternatively, you can incubate at 37 °C for 5 minutes)

- Place *immediately* on ice for 5 minutes.

- Add 200 µL SOC medium and mix gently by tapping the tube.

- Incubate at 37 °C for 2 hours.

- Warm up the LB agar plates to room temperature and label them. Make sure the plates contain the antibiotic that will select for your desired ligation product.

- Plate out 200 µL and 20 µL of the transformed cells onto two separate plates. Try to spread the cells evenly on the surface of the agar.

- Incubate plates at 37 °C overnight (12 - 14 hours) or at room temperature for 24 hours. Make sure plates are incubated with agar layer on top (and lid on the bottom).

- Plates with colonies can be stored at 4 °C (fridge) and can be used for at least a couple of weeks. Make sure plates are sealed with parafilm, labeled and oriented correctly (agar on top) while stored in the fridge.

5. Growing bacteria from agar stabs onto plates

The goal is to spread out a bacterial culture in order to get single bacterial colonies.

Materials

agar stabs with the bacteria you wish to grow	inoculating loop
LB agar plates	Bunsen burner
	incubator 37 °C

Protocol

- Obtain LB agar plates suitable for the bacteria you wish to grow. If bacteria carry plasmid DNA that makes them resistant to a certain antibiotic, use LB agar plates containing the respective antibiotic. For *E. coli* with no antibiotic resistance (e.g. NEB 10 beta or DH5 alpha cells), which you would need for making competent cells, use LB agar plates without any antibiotics.

- Warm up the plates to room temperature, and label them.

- Locate the sample of live bacteria inside the agar stab. Use an inoculating loop to scoop some of the bacteria and streak them onto a plate in a zig-zag line. Be gentle when drawing the line to avoid breaking the agar.

- Sterilize the loop, wait for it to cool and streak again starting from the end of the first line. This will spread out the bacteria further.

- Repeat previous step starting at the end of the second zig-zag line. This will spread out the bacteria even more.

- Repeat again, as needed.

- Incubate plates at 37 °C overnight (12 - 14 hours) or at room temperature for 24 hours. Make sure plates are incubated with agar layer on top (and lid on the bottom).

- Plates with colonies can be stored at 4 °C (fridge) and can be used for at least a couple of weeks. Make sure plates are sealed with parafilm, labeled and oriented correctly (agar on top) while stored in the fridge.

6. Growing up single bacterial colonies

The goal here is to obtain a large and homogeneous population of bacteria starting from a single cell. You need to follow this protocol when preparing stocks for later use (e.g. stocks of competent cells) or to check if the bacteria carry the correct (expected) plasmid DNA (see also section 5.6)

Materials

LB - agar plate with single bacterial colonies	inoculating loop

sterile LB broth (without or with antibiotic)	Bunsen burner
antibiotic stocks (if needed)	37 °C incubator and shaker

Protocol

- Use an inoculating loop to pick a single colony and add the bacteria to 5 - 10 mL LB broth. If needed, add antibiotic to the LB broth prior to inoculation (see table below).

Antibiotic	conc. of pre-prepared stock	final conc. in the LB broth	dilution factor	volume added to 5 mL LB broth
Ampicillin	100 mg/mL	100 µg/mL	1 : 1000	5 µL
Kanamycin	50 mg/mL	50 µg/mL	1 : 1000	5 µL
Chloramphenicol	50 mg/mL	25 µg/mL	1 : 2000	2.5 µL

- Grow overnight at 37 °C with shaking. Make sure the tube is capped loosely to allow air to enter the tube.

- In the morning, you can use the grown bacteria to make competent cells (see Making competent cells), or to isolate and purify the plasmid DNA (see Mini-prepping). Mini-prepping doesn't have to be done right away. Instead, you can store the bacteria at 4 °C (fridge) until you're ready. Make sure the tubes with bacteria are sealed tightly during storage.

7. Mini-prepping

To isolate and purify plasmid DNA from bacteria, you will likely use a commercially available kit such as E. Z. N. A. (Omega) or QIAprep (QIAGEN). Each kit comes with a handbook containing detailed protocols and troubleshooting guide (see also guidebook section 5.7). It is recommended that you read carefully the information in the handbook and that you follow closely the manufacturer's protocol.

The following protocol is based on the protocol from the OMEGA bio-tek handbook (E.Z.N.A. Plasmid DNA Mini Kit II). It is designed to produce ***40 - 75 µg plasmid DNA from 10 - 15 mL bacterial culture***.

Materials

solution I (with RNase A - see kit instructions)	centrifuge

solution II (warm at 37 °C to re-dissolve any precipitate)	centrifuge tubes
solution III (warm at 37 °C to re-dissolve any precipitate)	micropipettes and pipette tips
HiBind DNA mini column	2 mL and 1.7 mL microcentrifuge tubes
Equilibration buffer	microcentrifuge
HB buffer	spectrophotometer (optional)
DNA Wash buffer (with ethanol - see kit instructions)	
Elution buffer	

Protocol

- **HARVEST BACTERIA**: Pellet bacterial cells by centrifugation for 10 minutes at 4000 rpm. Make sure to keep about 1 mL of the overnight culture! This will allow you to grow up more cells and/or make a glycerol stock in case the bacteria contain the correct/desired construct.

- Pour the medium (the liquid on top of cell pellet) into a waste beaker. Be careful not to disturb the pellet. Any bacterial waste will need to be bleached or autoclaved before discarding!

- **RESUSPEND BACTERIA**: Add 500 µL solution I (with RNase A) to the cells. Pipet up and down vigorously to completely resuspend the cells.

- Transfer the resuspended cells into a clean 2 mL microcentrifuge tube.

- **LYSE BACTERIA**: Add 500 µL solution II. Mix gently by inverting the tube several times until you obtain a clear solution containing chunks of cellular debris. This step should not take longer than 5 minutes!

- **PRECIPITATE ALL CELL MATERIAL EXCEPT FOR DNA**: Add 700 µL solution III. Mix immediately by inverting the tube several times until a white precipitate forms and the solution turns cloudy.

- **CLEAR THE CELL LYSATE**: Separate the precipitate by centrifugation at maximum speed (14000 rpm) for 10 minutes. Transfer the *cleared lysate* into a new tube (if you are several minipreps at the same time, make sure to label the tubes clearly to avoid mixing them up).

- Place a HiBind DNA mini column into a 2 mL microcentrifuge tube. Pipet 100 µL Equilibration buffer on top of column and centrifuge at maximum speed (14000 rpm) for 1 minute. Discard the flow through liquid and re-place the column into the tube.

- **BIND NUCLEIC ACIDS TO COLUMN**: Pipet 700 µL of *cleared lysate* on top of column. Centrifuge at maximum speed (14000 rpm) for 1 minute. Discard the flow-through liquid and re-place the column into the same tube.

- Repeat previous step until all *cleared lysate* has been passed through the HiBind DNA mini column.

- **WASH OUT UNWANTED NUCLEIC ACIDS**: Pipet 500 µL HB buffer on top of column. Centrifuge at maximum speed (14000 rpm) for 1 minute.

- Discard the flow-through liquid and re-place the column into the same tube.

- **WASH OUT *E. coli* GENOMIC DNA**: Pipet 700 µL DNA Wash buffer (with ethanol) on top of column. Centrifuge at maximum speed (14000 rpm) for 1 minute.

- Discard the flow-through liquid and re-place the column into the same tube. You have the option of repeating the addition of DNA Wash buffer to improve plasmid yields.

- **DRY THE COLUMN**: Centrifuge the column at maximum speed (14000 rpm) for 2 minutes.

- Place the HiBind DNA mini column into a new 1.7 mL microcentrifuge tube.

- **RECOVER/ELUTE PLASMID DNA**: Pipet 80 µL Elution buffer on top of column. Wait for 1 minute then centrifuge at maximum speed (14000 rpm) for 1 minute. Under optimal conditions, this should result in a plasmid DNA concentration of 0.5 - 1 µg/µL. To recover any plasmid DNA left in the column, you have the option of repeating the elution step; however, this will produce a more dilute solution of plasmid DNA. DNA concentrations can be measured with a spectrophotometer or they can be estimated by running an agarose gel electrophoresis (the newly prepared plasmid DNA must be run side by side with a plasmid of similar size and known concentration).

- Store the plasmid DNA solution in - 20 °C freezer, or proceed immediately with setting up the restriction digestion.

8. Running agarose gel electrophoresis

This protocol is for 1% (w/v) agarose gels, which are suitable for many of the commonly used plasmids. To view small DNA fragments, you'll need a higher agarose concentration, while for large DNA fragments the agarose concentration must be lower than 1% (see also Methods section 5.8).

Materials

10 x TAE buffer	graduated cylinder
distilled water	stir rod
agarose	Erlenmeyer flask
DNA stain (e.g. Sybr Safe)	scale
DNA loading buffer 6 x	microwave
DNA restriction digest	tape

DNA ladder	apparatus for agarose gel electrophoresis (gel tray, comb, tank, power supply with connectors)
BioRad pen or blue-light transilluminator	micropipettes and pipette tips

Protocol

- Prepare 100 mL 1 x TAE buffer by adding 10 mL 10 x TAE buffer to a cylinder containing 90 mL distilled water. Mix well with a clean stir rod. This volume should be good for most gel cassettes.

- Pour the 1 x TAE buffer into an Erlenmeyer flask. There should be enough empty space left in the flask to keep the agarose from boiling over during microwaving.

- Weigh out 1 g agarose powder and add it to the 1 x TAE buffer in the flask.

- Place the flask inside the microwave and set at maximum heat for 1 minute. Pause the microwave every 10 - 15 seconds to give the flask a couple of swirls. This will help in dissolving the agarose and in preventing the solution from boiling over. Repeat as needed, until all agarose has dissolved. WATCH THE FLASK AT ALL TIMES AS IT MAY UNEXPECTEDLY BOIL OVER. NOTE: WEAR PROTECTION (OVEN MITTS) WHEN HANDLING THE HOT FLASK.

- Wait for the agarose solution to cool down but do not wait too long as it may solidify inside the flask.

- Assemble the gel cassette by sealing off the gel tray with tape. Place comb in proper location.

- Add 4 - 5 µL DNA stain (e.g. Sybr Safe) to the cooled agarose. Mix by swirling. Avoid forming air bubbles in the agarose.

- Pour the agarose solution carefully into the cassette. Avoid forming and trapping air bubbles.

- Wait for agarose to solidify.

- Prepare the DNA sample by mixing the following in a clean microcentrifuge tube to a total volume of 12 µL (see Note 1). ALWAYS INCLUDE THE DNA LADDER ALONG WITH YOUR SAMPLES. MAKE SURE TO ADD LOADING BUFFER TO THE LADDER, TOO.

2 µL 6 x Loading Buffer (see Note 2)

4 µL DNA restriction digest (see Note 3)

6 µL distilled water

- Peel the tape off the gel and remove the comb carefully.

- Place the tray with the agarose gel in the tank for electrophoresis. Make sure the wells are located at the negative end of the electrical field.

- Fill the tank with 1 x TAE buffer. Cover the gel with a layer of buffer 3 - 5 mm thick. If air bubbles get trapped inside the wells, displace them carefully using a clean pipette tip.

- Load your DNA sample(s) and the DNA ladder into the wells. Make sure to keep a record of which sample went into which well.

- Hook up the gel tank to the power supply. The wells with DNA must be on the negative (black) side!

- Turn on the power and run the gel at 100 V for at least 1 hour. The gel must stay covered throughout the run. ABSOLUTELY NO TOUCHING OF THE INSIDE OF TANK IS ALLOWED WHILE POWER IS ON!

- Switch off the power when the dye has travelled at least ¾ of the gel length and has neared the positive end of the gel.

- Unplug the gel tank. Take the gel out of the tank and view the DNA bands using a blue-light transilluminator or a BioRad pen. Take a picture of the gel and record the results.

Note 1 - The total volume can vary depending on the DNA concentration and the size of wells.

Note 2 - How much loading buffer is added depends on the concentration of the stock of loading buffer and on the total volume of the sample you load. The sample you load must contain loading buffer at an approximate concentration of 1 x. The loading buffer contains a dye which moves through the gel faster than any DNA and helps in tracking the run. It also contains glycerol which makes the DNA sink into the well.

Note 3 - You need to load 50 - 500 ng DNA in each well. If you expect to see just one or a couple of bands, 50 - 100 ng should be enough. If you expect more bands, and especially if they have smaller sizes, load 250 - 500 ng DNA. Here it is assumed that the DNA restriction digest has a DNA concentration of approximately 25 ng/µL (as in the protocol Restriction digestion of plasmid DNA), and two DNA fragments are expected. Therefore, approximately 100 ng DNA (4 µL x 25 ng/µL) are loaded.

9. Polymerase Chain Reaction (PCR)

Materials

sterile distilled water	sterile microcentrifuge tubes
10 x PCR buffer	micropipettes and sterile pipette tips
10 mM dNTPs mix	ice
10 µM forward primer	sterile PCR tubes
10 µM reverse primer	microcentrifuge (optional)
50 mM $MgCl_2$ solution	mineral oil
Taq Polymerase	PCR machine (thermo-cycler)
DNA template (s)	

Protocol

This protocol is for 5 PCR reactions of 50 µL each. It can be scaled up or down according to your needs.

- Obtain a sterile microcentrifuge tube and prepare *a master mix* by adding together ingredients as listed in the table below. Keep everything on ice, including the master mix. The total volume should add up to 225 µL (5 x 45 µL).

Ingredient	**volume for 1 PCR**	**volume for 5 PCRs**	**Final concentration**
sterile distilled water	35 µL	175 µL	-
10 x PCR buffer	5 µL	25 µL	1 x
10 mM dNTPs mix	1 µL	5 µL	0.2 mM
10 µM forward primer	1 µL	5 µL	0.2 µM - see Note 1
10 µM reverse primer	1 µL	5 µL	0.2 µM - see Note 1
50 mM $MgCl_2$	1.5 µL	7.5 µL	1.5 mM
Taq Polymerase	0.5 µL	2.5 µL	variable - see Note 2
	adds to 45 µL	adds to 225 µL	

- Obtain 5 sterile PCR tubes, label them and place them on ice.

- Pipet 5 µL DNA template into each PCR tube. The total amount of template DNA can vary between 1 pg and 1µg depending on the source (very little is needed if the template is pure plasmid DNA; significantly more will be needed if the template you use is chromosomal/genomic DNA)

- Add 45 µL of the master mix to each of the PCR tubes. Mix gently by tapping the tubes or by pipetting up and down a few times. If needed, spin down briefly at maximum speed in the microcentrifuge to collect all liquid at the bottom of tubes.

- Top the PCR reactions with mineral oil if your PCR machine doesn't have a heated lid.

- Transfer the tubes to PCR machine and start the program (you'll need to program your machine first):

STEP	TEMPERATURE	TIME

initial denaturation	95 °C	30 sec
30 repeating cycles	95 °C denaturation 55 °C annealing - see Note 3 72 °C extension - see Note 4	30 sec 30 sec - see Note 3 1 min per kb - see Note 4
final extension	72 °C	5 min
Hold	4 °C - 10 °C	

- When the program is over, you can proceed with the purification of the PCR product, which you can view by agarose gel electrophoresis.

Note 1 - The final concentration of primers can vary depending on the individual sequence and length.

Note 2 - The concentration/activity of *Taq* Polymerase varies depending on the source.

Note 3 - The annealing temperature and time will depend on the primers and will vary between 45 - 68 °C and 15 - 60 sec, respectively.

Note 4 - The temperature and time for extension will depend on the specific *Taq* Polymerase used.

10. Making glycerol stocks

You need to make glycerol stocks for long-term storage of bacteria carrying interesting and valuable constructs. You must have a - 80 °C freezer for this!

Materials

bacterial culture of interest	sterile microcentrifuge tubes (or screwcap tubes, if available)
sterile 80 % glycerol solution (mix 80 mL pure glycerol with 20 mL distilled water and autoclave)	micropipette and sterile pipette tips
	- 80 °C freezer

Protocol

- Obtain a couple of sterile microcentrifuge tubes and label them.

- Use a sterile pipet tip to transfer 0.5 mL of the bacterial culture of interest into each of the tubes.

- Use a sterile pipet tip to add 0.5 mL of an 80 % glycerol solution to each tube. Mix by pipetting up and down.

- Place in - 80 °C freezer for long-term storage.

11. Making agar stabs

You use this protocol when you don't have a - 80 °C freezer but you need to store bacteria over a longer period of time. Bacterial agar stabs can be stored for up to two years in the fridge (4 °C).

Materials

LB agar plate with single colonies of bacteria to be stored	microcentrifuge tubes (or screwcap tubes, if available) with sterile LB agar and the required antibiotic - **see Note**
37 °C incubator	sterile pipette tip or toothpick
	fridge

Protocol

- Obtain a tube containing sterile LB agar plus the required antibiotic.

- Using a sterile toothpick or pipette tip, pick a single colony and stab it into the agar a few times.

- Incubate the tube overnight (10 -12 hrs) at 37 °C making sure to leave cap slightly loose (but not completely open).

- Close the tube tightly and store it in the dark at 4 °C (fridge).

Note - It is best if these tubes are prepared at the same time as the LB agar plates used to obtain the single colonies of the bacteria to be stored. To do this you need to follow protocol 12 (Making LB agar plates) with the following modification: While the LB agar (to which you added the antibiotic) is still liquid, obtain a few sterile microcentrifuge (or screwcap) tubes and pipet 1 mL into each of them. Whether you do this before or after you poured the plates, you must make sure to work aseptically at all times! Cap the tubes and allow the agar to harden. Then store the sealed tubes at room temperature until needed.

12. Making LB agar plates

Materials

Tryptone	scale
Yeast Extract, bacteriological	glass bottle / Erlenmeyer flask
NaCl (Sodium Chloride, Molec Biol grade)	Petri dishes (sterile)
Agar, bacteriological	Bunsen burner
distilled water	aluminum foil
antibiotic stock solution (sterile) - if needed	autoclave tape
autoclave	0.2 - 1 mL micropipette and sterile pipette tips

Protocol

This protocol makes 500 mL LB agar for 20-25 LB agar plates. It can be scaled up or down according to needs.

- Weigh out and add the following into a 1 L glass bottle or Erlenmeyer flask:

5 g Tryptone

2.5 g Yeast Extract

5 g NaCl

7.5 g agar

- Add distilled water to 500 mL. The bottle must remain half empty to prevent the liquid from boiling over in the autoclave.

- Mix by swirling. Don't expect all solid powder to dissolve, but make sure that none gets stuck to the sides of the bottle.

- Cover the top with aluminum foil and secure it with autoclave tape. The cover must be loose enough to prevent pressure from building inside the bottle during autoclaving.

- Autoclave for 20 min at a pressure of about 15 psi.

- Remove from autoclave and allow it to cool to about 55 °C.

- If needed, add the required antibiotic. Swirl to mix. It is important that antibiotics are not added while the liquid is still hot because they get degraded. Make sure antibiotics are added aseptically! Use a sterile pipette tip and flame the opening of the bottle. Most common antibiotics are added according to information in the table below:

Antibiotic	conc. of pre-prepared stock	final conc. in the LB agar	dilution factor	volume added to 500 mL LB agar
Ampicillin	100 mg/mL	100 µg/mL	1 : 1000	500 µL (0.5 mL)
Kanamycin	50 mg/mL	50 µg/mL	1 : 1000	500 µL (0.5 mL)
Chloramphenicol	50 mg/mL	25 µg/mL	1 : 2000	250 µL (0.25 mL)

- Pour or pipette about 20 mL LB agar into each Petri dish. This must be done aseptically! Make sure to flame the opening of the bottle and to use a sterile pipette. Pour slowly to avoid introducing air bubbles.

- Place lids on Petri dishes and allow to cool and solidify. This will take at least 30-60 min.

- Invert the plates to avoid dripping of moisture onto agar. Label with antibiotic(s) name and date.

- Store LB agar plates at 4 °C (in the fridge) inside plastic bags or sealed with parafilm. If made and stored properly, plates will be good for at least one month.

13. Making LB broth

Materials and protocol are the same as for making LB agar plates with the following changes:

- No agar is added to the mixture.

- The autoclaved LB liquid (with or without antibiotics) is stored in the original bottle at room temperature or at 4 °C (in the fridge). Bottle must be sealed tightly to reduce the chance of contamination.

14. Making antibiotic stocks

Materials

antibiotic powder	scale
distilled water	15 mL polypropylene tube with cap

syringe	0.22 µm sterile syringe filter
sterile 1.7 mL microcentrifuge tubes	freezer (- 20 °C)

Protocol

This protocol is for 10 mL antibiotic stock. It can be scaled up or down according to your needs.

- Weigh out the necessary amount of antibiotic as indicated in the table below.

Antibiotic	Amount for 10 mL solution	Stock concentration
Ampicillin	1 g	100 mg/mL
Kanamycin	0.5 g	50 mg/mL
Chloramphenicol	0.5 g	50 mg/mL

- Add the antibiotic to 10 mL distilled water in a 15 mL polypropylene tube.

- Cap the tube and shake vigorously until all of the antibiotic dissolves and the solution becomes completely clear.

- Place 10 autoclaved microcentrifuge tubes in a rack.

- Draw the antibiotic solution into a syringe. Distribute the solution evenly among the 10 tubes by passing it through a 0.22 µm sterile filter. This will result in 10 x 1 mL portions (or aliquots) of sterilized antibiotic stock.

- Label and store the aliquots at - 20 °C (freezer).

15. Making competent cells

Because they carry no antibiotic resistance, competent cells can get easily contaminated.

It is important that you perform these protocols under strictly aseptic conditions!

15.1 Making fresh competent cells

Materials

NEB 10 beta or DH5alpha *E. coli* cells	inoculating loop

LB agar plate(s)	Bunsen burner
sterile LB broth	37 °C incubator and shaker
microcentrifuge tubes	sterile 10 mL and 15 mL tubes
filter sterile $CaCl_2$ solution (50 mM)	micropipettes and sterile pipette tips
ice	microcentrifuge
	spectrophotometer (optional)

Protocol

This protocol describes how to obtain competent cells for *immediate* transformation. You need to follow this protocol if you must do bacterial transformations, but do not have a - 80 °C freezer.

- Obtain a suitable stock of *E. coli* bacteria. For example, this could be an agar stab of NEB 10 beta cells or a suspension of DH5 alpha cells.

- Transfer some cells onto an LB agar plate and streak them out using an inoculating loop.

- Incubate plate overnight at 37 °C to obtain single colonies.

- Pick a single colony and use it to inoculate 5 mL LB broth (in a 10 mL tube). Grow overnight at 37 °C with shaking.

- In the morning, transfer 0.1 mL of the overnight culture into 10 mL LB broth (in a 15 mL tube).

- Grow at 37 °C with shaking for about 3 hours, or until the bacterial culture reaches an optical density OD600 of 0.4 - 0.6. The OD600 can be measured with a spectrophotometer.

- Transfer 500 µL (0.5 mL) bacterial culture into microcentrifuge tube and spin at 7000 rpm for 2-3 minutes to collect cells in a pellet. THIS WILL BE ENOUGH FOR ONLY ONE TRANSFORMATION. IF YOU MUST DO MORE THAN ONE TRANSFORMATION, YOU MUST SCALE UP. For example, for 4 transformations you'll need to spin down 4 x 500 µL portions of bacterial culture in 4 separate tubes.

- Discard/Remove the top liquid (supernatant) carefully to avoid disturbing the cell pellet.

- Resuspend the pellet in 500 µL sterile $CaCl_2$ solution (50 mM). Spin down again and remove the supernatant.

- Resuspend the cell pellet in 100 µL sterile $CaCl_2$ solution and place on ice. The cells are now ready to be transformed with plasmid DNA!

15.2 Making competent cells for later use

Materials

NEB 10 beta or DH5alpha *E. coli* cells	inoculating loop
LB agar plate(s)	Bunsen burner
sterile LB broth	37 °C incubator and shaker
microcentrifuge tubes	sterile 10 mL and 50 mL tubes
filter sterile $CaCl_2$ solution (50 mM)	200 mL Erlenmeyer flask
filter sterile $CaCl_2$ solution (50 mM, 15% glycerol)	micropipettes and sterile pipette tips
ice	microcentrifuge at 4 °C
- 80 °C freezer	spectrophotometer (optional)

Protocol

This protocol describes how to prepare stocks of competent cells that can be stored at - 80 °C to be used in later transformations.

- Obtain a suitable stock of *E. coli* bacteria. For example, this could be an agar stab of NEB 10 beta cells, or a suspension of DH5 alpha cells.

- Transfer some cells onto an LB agar plate and streak them out using an inoculating loop.

- Incubate plate overnight at 37 °C to obtain single colonies.

- Use an inoculating loop to pick a single colony and add the bacteria to 5 mL LB broth (in a 10 mL tube). Grow overnight at 37 °C with shaking. Make sure the tube is capped loosely to allow air to enter the tube.

- In the morning, transfer 0.5 mL of the overnight culture into 50 mL LB broth (in a 200 mL Erlenmeyer flask). Cover the flask to ensure aseptic conditions as well as air flow.

- Grow at 37 °C with shaking for about 3 hours, or until the bacterial culture reaches an optical density OD600 of 0.4 - 0.6. The OD600 can be measured with a spectrophotometer.

- Place 20 sterile microcentrifuge tubes on ice. These tubes must be cold by the time you put the competent cells in them. ALL SUBSEQUENT STEPS MUST BE DONE IN COLD (4 °C) AND ON ICE.

- Split the bacterial culture into two 50 mL tubes and spin at 3000 rpm and 4 °C for 20 minutes.

- Discard the top liquid (supernatant) and resuspend each cell pellet in 6 mL cold and sterile $CaCl_2$ solution (50 mM). Leave on ice for 10 minutes.

- Spin at 3000 rpm and 4 °C for 20 minutes.

- Remove supernatant and resuspend each cell pellet in 1 mL cold and sterile $CaCl_2$ solution (50 mM with 15% glycerol). Leave on ice for 30 minutes.

- Distribute competent cells in 100 µL portions (aliquots) into the pre-chilled microcentrifuge tubes.

- Store in the - 80 °C freezer.

16. Making 10 x TAE buffer for agarose gel electrophoresis

Materials

EDTA di-sodium salt	scale
distilled water	beakers
sodium hydroxide NaOH (solid or concentrated solution)	graduated cylinders
Tris base	stir rod or magnetic stirrer
Glacial Acetic acid	pH meter (or pH indicator paper)
	Clean bottles

Protocol

This protocol is for 1 Litre 10 x TAE buffer. It can be scaled up or down according to your needs.

To prepare 10 x TAE buffer *you must first prepare a solution of 0.5 M EDTA (pH 8.0).*

- Weigh out 93.05 g EDTA di-sodium salt.

- Add the EDTA to a beaker containing 400 mL distilled water. Mix using a magnetic stirrer or a stir rod.

- Adjust the pH of the solution to 8.0 by adding NaOH (EDTA will completely dissolve at pH 8.0).

- Add distilled water to a total volume of 500 mL and mix. Pour into a clean bottle for long term storage. Label the bottle < 0.5 M EDTA pH 8.0 >.

- Weigh out 48.5 g Tris Base and add to a beaker containing 800 mL distilled water. Mix to dissolve.

- Measure out 11.4 mL glacial acetic acid and add to the Tris solution.

- Measure out 20 mL 0.5 M EDTA (pH 8.0) and add to the Tris solution.

- Add distilled water to a total volume of 1 Litre. Stir to mix.

- Pour into a clean bottle for longer term storage. Label bottle < 10 x TAE >. Store at room temperature.

17. Making SOC medium

Materials

yeast extract	beakers
tryptone	pH meter or pH indicator paper
NaCl	stirring rod
KCl	1 L bottle
$MgSO_4$	autoclave
distilled water	autoclave tape
1M NaOH solution	sterile 10 mL tubes
glucose	syringe
sterile pipet	0.22 µm sterile syringe filter
micropipets and sterile pipet tips	graduated cylinder

Protocol

SOC medium is obtained by mixing SOB medium with 2 M glucose solution.

- To make 10 mL SOC, add 0.1 mL filter sterilized 2 M glucose solution to 10 mL sterile SOB. MAKE SURE TO DO THE MIXING UNDER STRICT ASEPTIC CONDITIONS!

17.1 Making SOB

- Weigh out the following ingredients and add them to a beaker containing 400 mL distilled water.

2.5 g yeast extract

10 g tryptone

0.3 g NaCl

0.1 g KCl

1.2 g $MgSO_4$

- Stir to mix. Adjust pH to 7.5 with a solution of 1M NaOH. Note: some of the solids will not dissolve.

- Pour into a 1 L bottle. Make sure to transfer any undissolved solid remaining on the sides of the beaker by rinsing the beaker with a little distilled water.

- Add distilled water to a total volume of 500 mL. The bottle must remain half empty to prevent the liquid from boiling over in the autoclave.

- Mix by swirling. Don't expect all solid to dissolve, but make sure that none gets stuck to the sides of the bottle.

- Cover the top with aluminum foil and secure it with autoclave tape. The cover must be loose enough to prevent pressure from building inside the bottle during autoclaving.

- Autoclave for 20 min at a pressure of about 15 psi.

- Remove from autoclave and allow it to cool. Label the bottle < SOB> and mark the date, seal it tightly and store it at room temperature.

17.2 Making 2 M glucose solution

- Weigh out 9 g glucose and add to a cylinder with 20 mL distilled water.

- Dissolve the glucose by stirring.

- Add distilled water to a total volume of 25 mL and stir.

- Pour the glucose solution into a clean beaker.

- Place 5 sterile 10 mL tubes in a rack.

- Draw the glucose solution into a syringe. Distribute the solution evenly among the 5 tubes by passing it through a 0.22 µm sterile filter. This will result in 5 x 5 mL portions (or aliquots) of filter sterilized 2 M glucose solution.

- Label and store the aliquots at - 20 °C (freezer).

Appendix 2.

DIY BIO

David Lloyd

1. What is DIY Biology?

Do-It-Yourself (DIY) biology is a new movement in the molecular biology world aimed at making molecular biology and genetic engineering more accessible. This idea was immediately embraced by the synthetic biology community for multiple reasons, the foremost being that it enabled access to people from outside the field. The DIY biology movement makes it possible for advanced technical skills in molecular biology to be effectively transferred into the hands of people who may have little to no speciality background.

Since high school students will typically enter the iGEM program with little speciality background knowledge, lessons from the DIY movement will likely come in handy when teaching students how to conduct molecular biology work. This section of the guidebook aims to teach you how to build molecular biology equipment, which you can use in your classroom to facilitate much of your iGEM wetlab (organism creation) work.

This section of the guidebook is to be used in conjunction with the ordering lists available. It is critical to identify early which materials you will buy, and which you will make yourself since there is a cost/time balance that needs to be incorporated into your project plan. For example, you could build a gel electrophoresis tank for little cost, however, sourcing the parts and building the unit may take several days without the promise of working in the end. Use your mentor and best judgement in determining a plan for what you should make using the tools in this section.

Please note: Many of these protocols may involve some work with electricity, power tools, or other potentially dangerous items. Please always follow strict safety guidelines, and if you feel uncomfortable performing any of the work described here please inform your iGEM mentors and they will be happy to advise you.

2. What is going on in the world surrounding DIY Bio?

DIY biology is already happening in many places around the world (see links below for some of the locations). For example Genspace, located in New York, is one of the largest DIY biology centres in the world. It has been functional for many years and acted as a powerful force in developing this movement. Another example is BioCurious, based out of the San Francisco area in California - a centre that opens up their doors to anyone interested in learning about synthetic biology. Be sure to look them up online if you are interested in learning more about these endeavours.

3. What kind of things do you need in your lab space?

In section 9 of this chapter is a list of equipment that your lab will need in order to get started with synthetic biology. Note that many of these materials may change depending on the specific needs and scope of your project. Use this list as a starting point and be sure to contact your mentors to help you identify the exact needs that your project will have.

Also noteworthy is that the links are not provided to tell you what you should actually buy; they are only supposed to give you an idea of the different distributors that exist for these products. The excel sheets that came with this list has a complete list of equipment needed, descriptions, and links to examples of what they look like.

4. What pieces of equipment can you build instead of buying?

You may find it useful to try and develop some of your own pieces of equipment rather than buying them. Below are some DIY projects that you and your students can undergo. Please communicate with your mentors prior to starting these projects as they may provide you with alternative solutions. There are five equipment solutions that are proposed in this manual, however, other resources are available online for making these pieces of equipment.

If you intend to do any of these projects yourselves, please make safety a priority. Some of the projects involve building electrical devices or using other potentially dangerous components. Please follow all necessary safety precautions when building or using these devices.

Potential DIY Devices:

1) Build your own Gel Electrophoresis set up
    a. Building a Power Source
    b. Building an Electrophoresis Gel Running Apparatus
    c. Gel Box solutions
2) Building Your Own Centrifuge
3) Building Your Own Incubator
4) Building Your Own Water Bath
5) Building Your Own Shaking Platform/Vortexer

Please note that the above list of equipment is a starting point, to show you some DIY opportunities developed by other individuals. It is recommended that you search (the internet for alternative ways to build these pieces of equipment prior to deciding on the most suitable way for you to do it.

5. DIY Building Projects

1) Building Your Own Gel Electrophoresis Set-Up

This protocol is for building a gel electrophoresis unit, a device which separates DNA through an agarose polymer. You can find more in regards to the use of this device in the molecular biology section of the guidebook.

a. Building a power source

The power source is the device, which will supply current to the gel box, forcing the DNA into the agarose gel and separating it. The device is basically a transformer, which will allow for a powerful voltage (usually between 80-100 V) to be applied to the solution. If you feel uncomfortable building it (with developing this unit), ask the mentors if there is any power source available to borrow.

One way to build a power source (there are a couple of different ways to make this device) can be found here: **http://www.instructables.com/id/Gel-electrophoresis-power-supply/step1/Materials/**http://www.instructables.com/id/Gel-electrophoresis-power-supply/step1/Materials/

http://www.instructables.com/id/Gel-electrophoresis-power-supply/step1/Materials/

An alternative way to build a power source is described below. The procedure was developed for a graduate student thesis at the University of Calgary. The goal of the thesis was to design a way to build a power supply, which could be used (constructed for use) in third world countries. The details are listed below:

Please note that all of this protocol was adapted from Dr. Leif Prebeau-Menezes Thesis with his permission:

Materials List:

Hammond Manufacturing Transformer [115 VAC 100VCT 0.5A]

PadBoard [~75 mm L x ~75mm W]

Male Pin Connector Receptacle [AC Inlet 2.8 mm]

Banana Jack (Red/Black) Female Plugs [2mm Sheathed]

Switch ON/ON [3- Way Receptacle Switch Rocker]

Low-Density Polyethylene(LDPE) or Polypropylene (PP) Non- Conducting Plastic Container [~220 mm L x ~130 mm W x ~120mm H]

Switch ON/OFF [2-Way Receptacle Switch Rocker]

Through Hole Resistors [RES 10.0K OHM 7W]

Capacitor [160V 47µF]

General Purpose Diodes

Standard Digital Multimeter [Volt/Ampere/Ohm meter]

Standard Glue Gun and Glue Sticks [10W Dual-Temperature]

Standard Soldering Iron [60W Pencil Tip] and Solder [63/37 20 Gauge 1/2 lb]

Receptacle, Female Pin Connector with 3 Pin Electrical cord [6 Inches in length]

Hook-up Wire (Red/Black) [300 V]

Retractable Utility Knife with Snap off Blades

Procedure:

1) In the clear, non-conducting, LDPE/PP plastic container four holes of approximately 6.0 mm in diameter were cut with a sharp retractable utility knife at approximately 30 mm from the corners of the container. The top two holes on the container were used for installing the black female banana jack plug (negative outlet lead) and the red female banana jack plug (positive outlet lead), identified as "A" in Figure 1.

The bottom left hole, identified as "B", in Figure 1, was used for mounting the toggle switch between 70V and 140V (ON/ON 3- Way Receptacle Switch Rocker). The bottom right hole, identified as "C", in Figure 1, was used to install the main ON/OFF power switch (2-Way Receptacle Switch Rocker). Two green peg-boards, recycled from discarded pipette boxes, were used as reinforcement for the container.

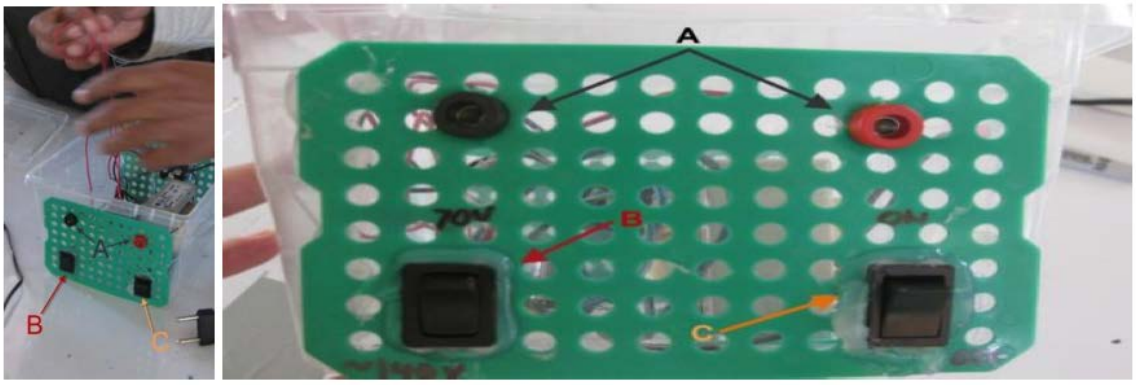


Figure 1: Illustrates the location of: A) banana jack red/black female plug set; the black negative plug on the left and the red positive plug on the right, B) toggle switch for switching between 70V and 140V, and C) main ON/OFF power switch

2) On the opposite side of the container, a single hole approximately 30mm L x 15mm W was cut with a retractable utility knife and was prepared for the installation of the male pin connector receptacle (AC Inlet 2.8 mm) identified as "A", in Figure 2.

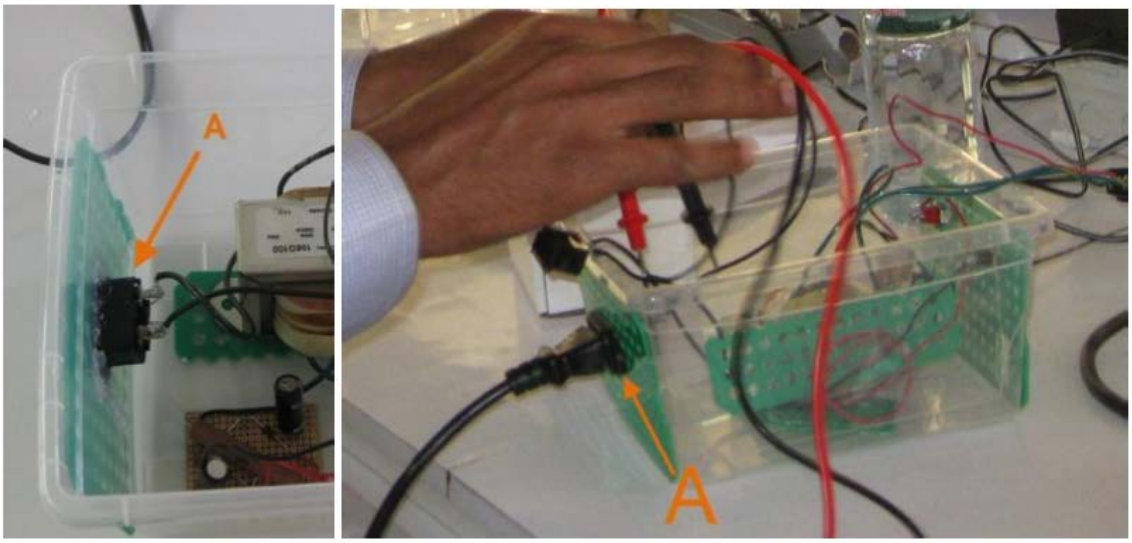


Figure 2: Illustrates the location of: A) the male pin connector receptacle attached to the clear LDPE non-conducting container

3) The Hammond Manufacturing Transformer (115 VAC 100VCT 0.5A) and electronic components were configured to the Padboard according to the circuit diagram (Figure 3). All electronic components, wiring and circuit board connections were soldered using a standard soldering iron (Standard Soldering Iron 60W Pencil Tip) and solder. With this current configuration it was imperative that only 110V AC inlet power be supplied to the transformer. Any current above this

threshold would damage the electrical components, the transformer, and become a potential fire hazard rendering the unit inoperable.

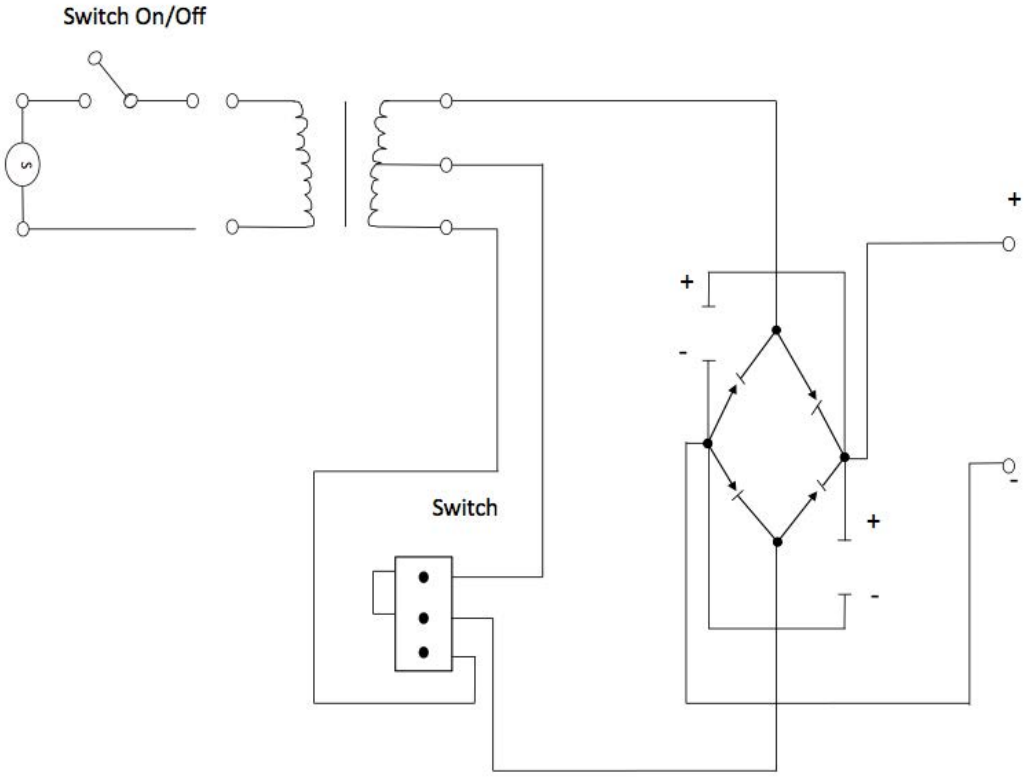


Figure 3: Circuit Diagram

4) The transformer, padboard with its electronic components, positive and negative leads, switches and male pin connector were securely mounted onto the LDPE/PP 30 container as shown in Figure 4, using the non conductive plastic adhesive from the glue gun (Standard Glue Gun 10W Dual-Temperature with glue sticks).

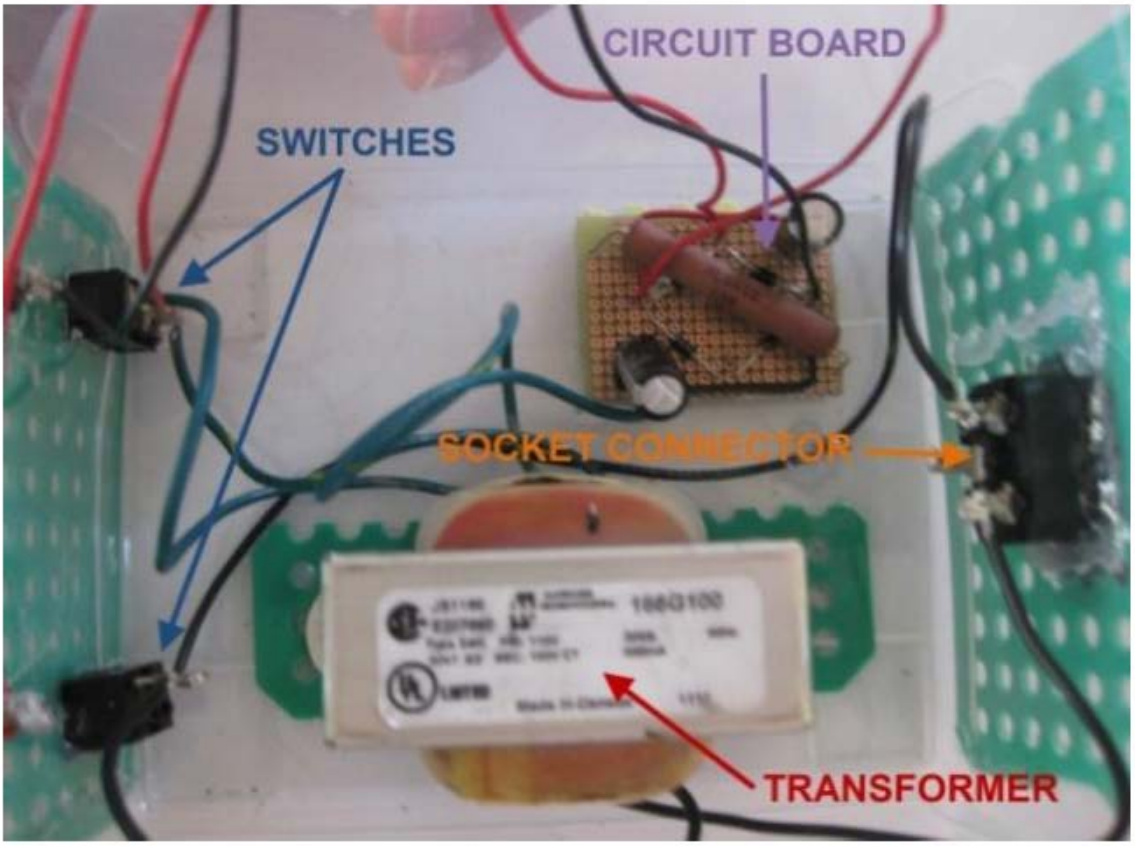


Figure 4: Illustrates the electronic components, positive and negative leads, switches and male pin connector receptacle mounted to the container securely using non conductive plastic adhesive

5) The cover of the container was placed securely to prevent accidental opening or tampering. The outside of the container was labelled PLUG IN TO 110V ONLY to prevent accidental plug-in to the lab-electrical input connection of 220V AC (Figure 5).

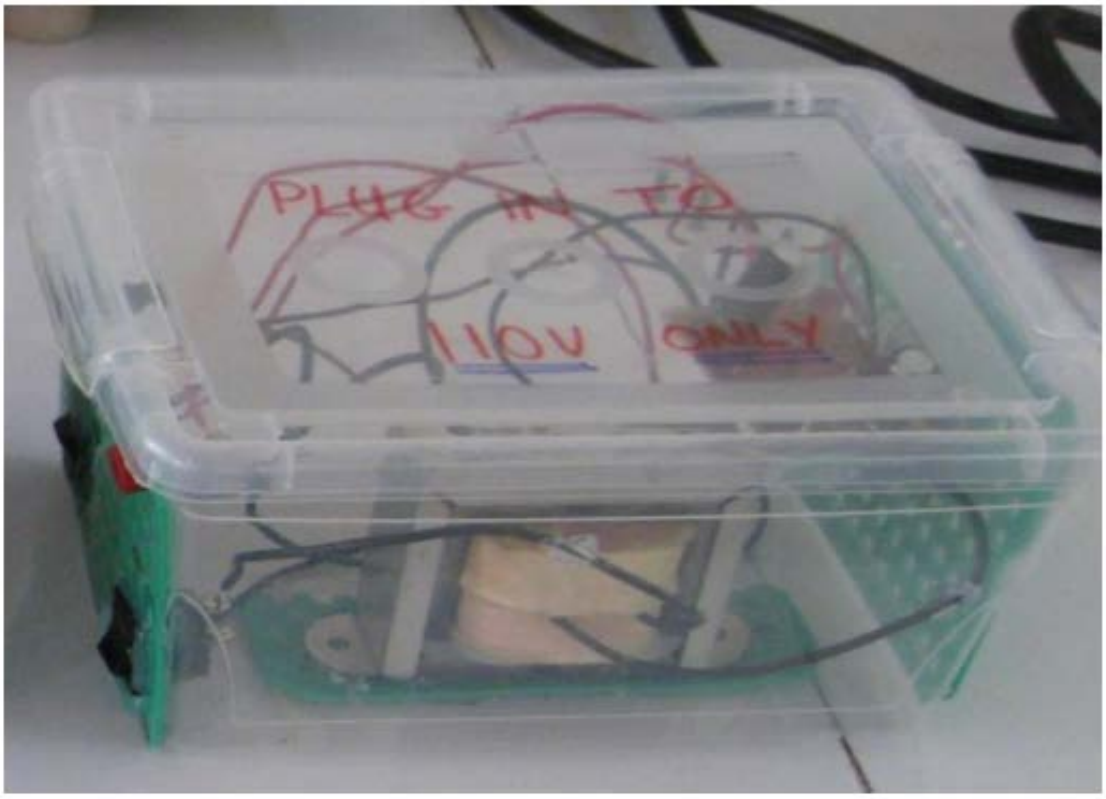


Figure 5: SBPSU with secured cover and warning label indicating only 110V AC input

Construction Safety Protocols:

The safety protocols observed during the construction of the SBPSU were:

1) Ensuring that the equipment chassis and cabinets were grounded.

2) Disconnecting power and unplugging all unnecessary electric equipment before working on the electronic circuits.

3) Avoiding any contact with moisture or water during construction. Never placing containers of liquid on/near the SBPSU.

4) Removing metal ornaments such as jewelry, watches, rings, etc., before working on electrical circuits.

5) Ensuring to never overload circuits or leave unprotected (charged) SBPSU's unattended.

6) Securing the SBPSU properly for extended absences/storage.

7) Safely discharging capacitors in the SBPSU before working on its circuit board.

8) Wearing proper personnel protective equipment before doing any soldering or working alongside an open flame.

b) Building a gel electrophoresis tank

Once you have a power supply (voltage pack), you can hook it up with the gel electrophoresis tank to perform your experiments. You can make a gel electrophoresis tank fairly easily. However, because of the safety considerations involved, if you do not feel absolutely comfortable with building it yourself, it is recommended that you purchase a professionally built electrophoresis unit instead.

Protocol 1: This is a very crude design, but it gives a great overview of how the technique works and what you will need for building one of these devices. Note that it is much safer than some of the other types of constructions. If you already have a voltage pack, it is not as important that you use this kind of design: http://www.sciencebuddies.org/science-fair-projects/project_ideas/BioChem_p028.shtml#procedurehttp://www.sciencebuddies.org/science-fair-projects/project_ideas/BioChem_p028.shtml - procedure

http://www.sciencebuddies.org/science-fair-projects/project_ideas/BioChem_p028.shtml - procedure

Protocol 2: This contains very clear instructions on how to build a gel box (one of these containers). Note that the wires used are bare and therefore they pose more of a safety concern. Using plastic coated wires instead (this) will decrease the risk associated with using the device:

http://citizensciencequarterly.com/?p=3084&preview=truehttp://citizensciencequarterly.com/?p=3084&preview=true

http://citizensciencequarterly.com/?p=3084&preview=true

Once you have the power supply and the gel tank you can run a DNA agarose gel. After running the gel, you will need to visualize the DNA inside the gel. One of the easiest ways of doing this is with SyberStain, which will allow low UV or blue light to visualize the DNA very well. A blue light pen, available for purchase from BioRad, will allow you to visualize the DNA:

Long-Wave UV Pen Light #166-0530EDU
http://www.bio-rad.com/en-us/sku/166-0530edu-long-wave-uv-pen-light

For some applications, using a blue light pen may make it difficult to see the DNA, another alternative to using a light box is to use a black light. These can be bought from most retailers and provide great UV light for visualizing the DNA. The stronger the blacklight the better (short wave UV light works better than long wave UV light).

2) Building Your Own Centrifuge:

The following excerpt and procedure were taken from:

http://www.thelabworldgroup.com/blogs/build-your-own-centrifuge-under-fifty-dollars-diy-centrifuge-project

< The 3-D–printed wheel looks deceptively simple, with six slots for standard 1.5-microliter Eppendorf tubes, oriented horizontally. Garvey says his early prototypes often deformed, which cracked and shot off tubes like oversize plastic bullets. "People said I was mad," Garvey says. Yet he prevailed, reshaping the wheel's slots to safely cradle the tubes' thick rims. "I've never had a tube eject since," he says. Still, Garvey advises using extreme caution—and proper eye protection—when running what he's dubbed the Dremelfuge.>

The procedure will require access to a 3D printer. You may have to do a bit of searching to see if you can find a 3D printer in your area. There is one available at the University of Calgary.

Procedure:

1) Download schematics for the Dremelfuge wheel here (https://gitorious.org/dremelfuge). Two designs are available; pick the one that fits a Dremel 300 (not a standard drill).

2) 3-D–print the wheel in ABS plastic, using hexagonal infill to strengthen it. (PLA is another common printing material, but Garvey says it tends to shatter under high centrifugal G-forces.) If you lack a 3-D printer, order the wheel from Shapeways.com for about $50.

3) Screw a rotary-tool disc holder into the wheel's axis, and attach it to a Dremel.

4) Fit tubes containing biological samples into the wheel, making sure that each tube has a counterweight for balance. This means that there is always another tube on the opposite side holding an equal amount of liquid. Failure to do this will lead to damage of the centrifuge and a big mess.

5) Place the Dremelfuge in a Styrofoam cooler, and let 'er rip! The foam will absorb the impact if a tube pops off.

***WARNING:* Use shatterproof eye protection, and operate the Dremelfuge inside a sturdy container, as the device can throw off hunks of plastic at dangerously high speeds.**

TEST RUN of your newly built centrifuge

The cells lining your inner cheeks constantly slough off into saliva but are too small to see individually—This makes them perfect candidates for testing the Dremelfuge.

1) Swab a Q-tip inside your mouth for about 10 seconds to grab some inner cheek cells.

2) Dip the cotton tip repeatedly in a tube filled with isotonic saline solution to dislodge the cells. (Make your own 0.90 % w/v solution from table salt and distilled water, or buy wound wash at a pharmacy.)

3) Fill a second tube with water to the same level as the one containing the cells. Insert the tubes in opposite slots in the 3-D–printed wheel.

4) Run for one minute at the Dremel's second setting (about 10,000 rpm). You'll see a whitish cell pellet at the bottom of the tubes. Voilà! Isolated cheek cells.

3) Building Your Own Incubator:

Again there are multiple strategies for how to build your own incubator. One of the methods is detailed below. You can also use any of the set-ups for chicken eggs incubators available online. Your incubator must hold the temperature constant at or near 37 degrees Celsius for growing *E. coli*. The closer you keep it to that temperature the better your bacteria are going to grow.

The following was taken from "1999 Science in the Real World: Microbes in Action". The material may be duplicated by teachers for use in the classroom; however any other use is prohibited.

Materials

- 20 gallon aquarium (does not need to be watertight)
- Heavyweight clear plastic
- Strong tape
- Small lamp that can use up to 75-watt bulb
- Thermometer (0-100º C). (preferably in a clear plastic thermometer case)

Instructions

1. Turn the aquarium so that the opening faces the front instead of the top.

2. Cut the plastic slightly wider than the opening and about 2 inches longer than the height of the opening.

3. Tape the plastic to the top of the aquarium, so that the plastic falls over the opening at the front. This is your "door."

4. Place the lamp in the aquarium, letting the cord come out the front under the plastic covering.

5. Place the thermometer in the aquarium so that you can take readings through the glass without opening the plastic "door."

6. Try different bulbs until you find one that gives you the temperature you need for your incubator.

You can be really fancy and attach a dimmer to the lamp so that you can regulate the temperature by using the dimmer switch.

A genetic engineering experiment using E. coli grown inside this kind of incubator is described by John Iovine in "The Amateur Scientist" in Scientific American, June, 1994 pp. 108-111

4) Building Your Own Water Bath:

Only the link to how to build one of your own water bath units will be provided here. The online instructions are great but do require some ability to solder your own electronics. The water bath

described controls temperature accurately (It is very accurately able to control water temperature) and is quite cheap. If you do not presently have a water bath unit, this may be a cheap viable option for you. There are numerous other alternative procedures for being able to build a water bath that you can find by searching the internet. If you want to use another procedure, be sure to talk to your mentor about safety.

http://seattlefoodgeek.com/2010/02/diy-sous-vide-heating-immersion-circulator-for-about-75/http://seattlefoodgeek.com/2010/02/diy-sous-vide-heating-immersion-circulator-for-about-75/

http://seattlefoodgeek.com/2010/02/diy-sous-vide-heating-immersion-circulator-for-about-75/

5) Building Your Own Shaker and Vortex Mixer:

Vortex mixer units are used for mixing solutions rapidly. A vortex can be purchased at a fairly cheap price and it can be adapted for mixing multiple solutions at the same time using the following protocol. It could also be used in conjunction with an incubator to heat solutions at low speed and 37 degrees Celsius.

http://citizensciencequarterly.com/2012/01/cheapass-science-diy-vortex-mixer-tube-holder/http://citizensciencequarterly.com/2012/01/cheapass-science-diy-vortex-mixer-tube-holder/

http://citizensciencequarterly.com/2012/01/cheapass-science-diy-vortex-mixer-tube-holder/

6. Other DIY Opportunities

1) Solutions to an autoclave:

Autoclaving is a process by which you heat a solution or substance at a high temperature and pressure. Normally, it uses steam at about 121 degrees Celsius for 20-45 minutes, which is enough to destroy all organic contaminants in a solution. Autoclaving is needed primarily to destroy spores that are otherwise hard to kill off using regular antibiotics or cleaning reagents.

If obtaining an autoclave is difficult for you, here are some tricks to get around this issue. An important thing to remember is that you need about 30 psi - pounds per square inch of pressure - in order to achieve proper sterilization. One option is to use a **pressure cooker in order to sterilize media and other components you will be working** with. Remember that the pressure cooker must reach a psi level of 30.

Whenever you autoclave something be sure to place a piece of autoclave tape onto the item. This special tape will turn black if sufficiently high temperature and pressure is reached to kill off bacteria and other organic contamination.

One component that you will need to prepare often is the nutrient solution that your cells can grow in (LB Broth – see protocols section for more detail). Since this will be one of the most common solutions that you will use, it is convenient to know ways to get around having to autoclave it. Here is a possible protocol to use:

1. Add 32 grams of LB Agar powder to a 1 L autoclaved/sterile bottle.

2. Using a clean and autoclaved/bleached graduated cylinder, fill up bottle with 900 mL of distilled water (if you use a graduated bottle you can pour the distilled water directly inside the bottle).

3. Close the cap and shake vigorously.

4. Remove cap (!) and microwave on High for 1 min. Repeat this several times, stirring occasionally, until the LB Agar is dark yellow and slightly viscous and there are no lumps left. Be sure to check the bottle often as it may boil over!

5. Place bottle in 50 degree Celsius water bath with the cap loosely fastened. Stir gently after 30 minutes.

6. The LB Agar is ready to pour into plates when the bottle is slightly warm and easily handled.

7. Thaw out a tube of Ampicillin.

8. Add the Ampicillin to your bottle of LB Agar (see protocols section for amount of antibiotics to add). Stir with a sterile pipette or gently twirl the bottle to obtain even mixing.

9. Lay out 50 petri dishes (2 sleeves) on the bench top; keep the plastic sleeves. You can choose to either use a pipette aid to pipette your LB Agar, or you can pour directly into the plate. Pour about ¼ inch of LB Agar per plate.

10. Cover petri dish with the petri lid; be sure to leave the lid slightly off center until Agar is fully cooled and hardened.

11. After the plates have cooled and solidified, replace them in to the plastic sleeve and close with some tape. Be sure to label the tape with "LB Agar + Amp" and the date the plates were made.

NOTE: THIS PROTOCOL IS NOT FOR MAKING LB AGAR PLATES WITHOUT ANTIBIOTICS!!! If you need LB agar plates without antibiotics, make them the old fashion way using the autoclave (trust me, you'll be sorry if you don't).

2) Solutions to a -80 degrees Celsius Freezer:

In order to store cells, certain solutions, and other chemicals for long periods of time you require a freezer, which can reach -80 degrees Celsius. These are expensive and difficult to obtain. Therefore you may need some solutions to getting around using this piece of equipment.

One major reason why you will need a -80 degrees Celsius freezer is to store competent cells. These are cells ready to be used for transformation, which is the process by which cells take up foreign DNA. The procedure in the protocols section of the manual describes a way to prepare competent cells fresh every time you need them to avoid the need for a freezer to store them. Please see this protocol for how to get around using a -80 degree freezer.

The bacteria successfully transformed with new DNA constructs must be placed in long-term storage. This is done by preparing a glycerol stock of these bacteria. A glycerol stock is a suspension of the cells in LB media with 30% glycerol, which allows them to stay alive at temperatures as low as -80

degrees Celsius. If you have no access to a -80 degrees Celsius freezer you have several alternative options:

Create an agar stab

Take a 15 mL Falcon tube (sterile) and pour in liquid LB Agar with the appropriate antibiotic selection for your construct about half way. Take some of the bacterial culture that you wish to preserve and stab it into the LB Agar once it has solidified and cooled. Store this in a fridge with the cap tightened. These cells will usually be good to use for a fairly long time. For more specific instructions see the appropriate part of the methods section.

Store you glycerol stocks at -20 Degrees Celsius i.e. in a regular freezer

Follow the procedure in the protocols section but store the tube in the freezer. These cells will not be as stable as when stored at -80 degrees and they can lose the new construct over time. This method is only recommended as a backup way of storing your cells.

Alternative Room Temperature Storage Solutions

There are companies that offer cryo-solutions using new and innovative ways of storing cells at room temperature. You can check them out online by searching for "room temperature cryo solutions for bacterial long term storage".

3) Solutions To Measuring Number of Cells

You will very likely need to have a method for measuring the number of cells in a cell suspension. This is usually accomplished with a spectrophotometer by measuring the optical density (OD) of the suspension at a wavelength of 600 nm. You can do a google search to learn more about what the spectrophotometer does or see the equipment list for examples. The problem is that a spectrophotometer can be expensive! An inexpensive way of getting around a spectrophotometer is by using a mini-spectrophotometer on your iPhone or Android device!

http://shop.breadpig.com/products/mini-fold-up-spectrometerhttp://shop.breadpig.com/products/mini-fold-up-spectrometer

http://shop.breadpig.com/products/mini-fold-up-spectrometer

7. **Where to Buy Equipment That You Cannot Make**

Most equipment can be purchased from large biotechnology companies that make top of the line equipment for premium prices. On a limited budget you may not be able to afford all of the top end equipment that you need. Some of the main companies that sell biotechnology equipment are:

Thermo-Fisher Scientific – Equipment and Reagents

Bio-Rad – Equipment and Reagents

VWR – Equipment Reagents, Chemicals, Consumables

Life Technology (Invitrogen a division of Life Technologies) – DNA synthesis, Equipment, and Reagents

Roche – Some consumable and reagents

Rose Scientific - Equipment

Sarstedt - Consumables

Sigma Aldrich – Consumables and Reagents (mainly chemicals)

NEB Biosciences – Molecular Biology Reagents (specifically enzymes, ligase, etc.)

Cedar Lane – Distribution of Consumables and Reagents in Canada

Gold Biotechnology

Be sure to check out these companies' websites to get an idea of the types of high-end equipment that are available. You will notice that many of these companies do not list prices for their equipment as the selling price will vary depending on the institution that you are with. If you are thinking to buy something from one of the companies listed above please contact the mentors as they may be able to get you a discounted price by using university pricing.

Some of these companies will service high schools, particularly NEB Biosciences, which is a great resource for the enzymes you will need, as well as Sigma, from which you can purchase many of the chemicals you will need.

VWR, Fisher, and Cedar Lane are distribution companies that sell products from various other biotechnology groups and are great resources for alternative products available on the market.

Alternative vendors that you can use are second hand refurbished providers such as www.southwestscience.com. Such companies sell biotechnology products at a lower price compared to other biotechnology companies; however, the disadvantage is that they don't guarantee the quality of the machinery that they are selling to you. This is just one provider and there are numerous others in North America. It is highly recommended that you shop around (on the internet) with as many providers as possible to find the best fit (that is best) for you.

8. Other References

a. diybio.org/
b. https://groups.google.com/forum/#!forum/diybiohttps://groups.google.com/forum/#!forum/diybio
c. http://openwetware.org/wiki/Protocolshttp://openwetware.org/wiki/Protocols
d. http://openwetware.orghttp://openwetware.org
e. http://openwetware.org/wiki/Materialshttp://openwetware.org/wiki/Materials
f. http://openwetware.org/wiki/Tk:lab_stuffhttp://openwetware.org/wiki/Tk:lab_stuff
g. http://www.mlo-online.com/articles/201112/the-quest-for-the-500-home-molecular-biology-laboratory.phphttp://www.mlo-online.com/articles/201112/the-quest-for-the-500-home-molecular-biology-laboratory.php
h. http://www.popsci.com/diy/article/2013-07/how-build-your-own-diy-centrifuge

9. List of Materials and Equipment

Equipment - Small Items, Miscellaneous and Consumable Items

Item Name	Type	Descripition	Links and Additional Information
Timer	Accessory	For timing incubations	
Tube holders	Accessory	For holding tubes. Can be made from styrofoam if needed, don't spend money on this if you can.	http://alkalisci.com/tube-racks.html
Ice buckets	Accessory	Often just use potted plant containers or styrofoam boxes. Do not buy professional grade ice buckets as they are costly and not necessary.	
Calculator	Accessory	Needed for math calculations	
Lab Notebooks	Accessory	Anything you do must be recorded in a laboratory notebook. Please read specifics on how to properly document a laboratory notebook prior to performing experiments. It should be a hard cover book that is always written with pen.	
PCR tube holders	Accessory	Based on your PCR machine you will either need 0.2 mL or 0.6 mL volume. This item is only needed if used with a PCR Machine. Talk to your mentor about the size of tubes that you will need to buy and the appropriate holder for these tubes	
Test tube racks	Accessory	Used for holding test tubes or the culture tubes needed to grow bacteria.	http://www.amazon.com/b?ie=UTF8&node=318133011
Floaters	Accessory	Plastic pieces with holes in it for holding tubes in the water bath. These should hold 1.5 mL tubes and should float.	
Spreader	Accessory	A flat ended piece of metal required for spreading bacteria when plating onto agar	http://www.labsource.com/Catalog/Group.aspx?GroupID=1781
Wash bottles	Accessory	Used for holding ethanol, water, or soap in a laboratory setting	
Tweezers	Accessory	Used for picking up small things	
Autoclave Gloves	Accessory	Padded gloves for grabbing hot liquids (only necessary if you have an autoclave) The liquids may be close to 100 degrees Celcius	
Spatula	Accessory	For weighing out chemicals. Can also use spoons.	
magnetic stir bars	Accessory	For mixing solutions to be used in conjunction with the stir plate (see large equipment)	
Sponge	Accessory	For the safety kit	
Mop Bucket	Accessory	For the safety kit	
Spill Kit	Safety	This is required for safety. Its contents can be found under the safety section of the guidebook	
Plastic Bins	Accessory	Used for storage of tubes, tips, etc. If it is strong plastic you can autoclave it. Not all plastic is autoclavable! If you are unsure it is recommended that you talk to you mentor!	
Pipette Bulb	Accessory	Used with the serological pipettes to suck up liquid.	
Bunsen burner	Accessory	Needed to sterilize tubes for transfer of liquid or other material. Used with your gas line.	
End over end generator	Accessory	A rotator piece of equipment. Not completely required but can be handy if you need to be mixing something continuously. This is also something that you can put together yourself quite easily.	
Glass bottles	Accessory	1L and 500 mL Glass Bottles are most common	
Beakers and Erlenmeyer flasks	Accessory	Various sizes. Should hold 100 mL - 500 mL range. Good to have multiples of each of them.	
Thermometer	Accessory	Used to measure temperature of your water bath, fridge, freezer, etc.	
Eppendorf tube holders	Accessory	These will be the most common plasticware you will use. Buy holders for 1.5-1.7 mL tubes. These have several names but we often refer to them as Eppendorf tubes or as 1.5 mL tubes. Do not confuse these with the smaller tubes that can sometimes be used for PCR applications.	
Grad cylinders	Acessory	50 mL, 100mL, 250 mL, 1L. Plastic containers for measuring out liquids in your lab space.	
Hand soap	Consumable	Buy from a grocery store. For use to wash hands only.	
Disposable Gloves	Consumable	Make sure everyone is always wearing gloves. See biosafety. You can buy latex or nitrile based gloves (either work fine) ensure no one in the lab has a latax allergy if using this glove type	
Paper Towel	Consumable	For washing hands. Make sure it is not used with any chemicals. Also good for cleaning spills and should be found in your spill kit.	
15 mL Centrifuge Tubes	Consumable	For growing and spinning medium-volume bacterial cultures. We sometimes call this a 15 mL Falcon tube because that is the name of the brand.	
50 mL Centrifuge Tubes	Consumable	For growing and spinning large volume bacterial cultures. We sometimes call this a 50 mL Falcon Tube, because that is the name of the brand.	
Toothpicks	Consumable	For picking colonies and growing them in liquid media. Make sure that they are autoclaved prior to use. An alternate to this is to just used autoclave pipette tips to pick up bacterial colonies and drop the tip into liquid media to start growing them.	
Parafilm	Consumable	Stretchy plastic wrap like material for sealing tubes, plates, etc. It will change your life!	
Alcohol for spreader	Consumable	You will need 70% ethanol, but you can buy it at higher concentration. Note that if your school has not dealt with ethanol before it may be tricky to get started as this is a controlled substance. Contact your mentor if you have any concerns with the use of ethanol in your lab space.	
Razor Blades or Scalpels	Consumable	For cutting into gels. Do not need to be sterile but should be clean. Be sure to dispose of correctly see biosafety.	
Pens	Consumable	Basic office supplies	
Lab markers	Consumable	Basic office supplies	
tape	Consumable	Basic office supplies	
stapler	Consumable	Basic office supplies	
scissors	Consumable	Basic office supplies	
autoclave tape	Consumable	Tape which turns black after it has been autoclaved - to confirm proper autoclaving of materials. Be sparing with your autoclave tape, you only need a small amount of it to see it change colour	
0.2 um filters	Consumable	It is important that the size of the filter be 0.2 um. This is below the size of bacteria and other microorganisms so it can be used to sterilize solutions. This must be used with a syringe.	
plastic (disposable) syringes	Consumable	Needed for using the filters to be able to filter solutions. Remember that there are two ways to sterilize everything you will be using. For small volumes (100 mL or less) you can use a filter to sterilize the solution. For anything larger you should autoclave it. Remember all filtered solutions must go into a sterile container!	http://www.medical-and-lab-supplies.com/syringes-needles/syringes-only.html
lab tape	Consumable	For labeling tubes. An alternate to biotech lab tape is masking tape.	
petri dishes	Consumable	Must be sterile! For making agar plates to grow your bacteria on. Typically should order a case of these as you will go through petri dishes quite quickly. An example of the type of petri dish you can use is in the sample order list.	
weigh boats/paper	Consumable	For weighing out materials on your scale. Weigh boats can be very expensive. You can always buy wax paper, or another non-stick paper type of material to use for weighing out materials. Use your best judgement based on the type of scale that you have and access to different materials. Talk to your mentor with any questions.	
pH Strips	Consumable	Make sure you have pH strips that allow you to read between 3 - 10 pH level. This can be used instead of the pH Meter that was proposed in the large equipment order.	
Bleach	Consumable	Used to sterilize surfaces or solutions which contained bacteria. See biosafety. Buy this from a grocery store.	
Kimwipes	Consumable	Non-scratch tissue paper good for cleaning tubes or glass. See sample order list for an example.	
serological pipettes 5, 10, 25 mL	Consumable	Long pipettes which make it easy to measure volumes from about 1 mL to 25 mL volumes. They require a pipette bulb. See the link for an example of what these look like. If you plan to use them with bacteria, make sure that you order sterile ones! Plastic are much less expensive than glass, however, glass can be autoclaved and used again if you have a way to store them. See the Sample order list for an example of where you can buy these	http://www.globescientific.com/serological-pipettes-c-28.html?gclid=CjwKEAjw9qafBRCRiYrL4-fpuFkSIACvocQ1Km3nbdEQUMHDHbFOGhKJ02JFK1g5KeQJcf6_VhSoCBoCv-Hw_wcB
pipette tips	Consumable	Pipette tips are needed for each of your pipettes. Remember that each pipette has a unique type and style of tip that you will need. See the consumables samples order list for an example of the type of pipette tips you can order. These are to be used with the brand of micropipettes that you order from the large equipment list.	
aluminum foil	Consumable	Aluminum foil can be autoclaved, so it can be used to wrap bottles, or other containers and seal them. This can be bought at a grocery store.	
Flint	Consumable	Needed for the flame cracker to start the the bunsen burner. May not be required if you do not use a flame cracker.	
Flame cracker	Consumable	Used to start the bunsen burner. You can use a lighter, however, please be very careful when using any other mechanism of lighting a flame using a gas line and bunsen burner.	
Plastic pipettes	Consumable	Disposable eye dropper styled pipettes for addition of liquids into other solutions. See the link for an example.	http://www.2spi.com/catalog/spec_prep/other2.shtml
Sharps disposal bin	Disposal	For now, you may want to just use a labelled plastic container, but sharps need to be disposed of in a particular way. This involves a container that has a tight sealing lid. Talk to the individuals responsible for disposal at your school or your mentor on how to appropriately deal with sharps waste.	
Plastic autoclavable containers for tubes	Disposal	This will be used for waste materials once you have finished using them. The tubes will be collected in this container and you can autoclave the waste to despose of all of the waste at a later date. See the dispoal section for more information.	
Lab coats	Safety	Everyone should always be wearing a lab coat in the lab for safety. Read the safety section for more details.	

Equipment List - Large Items	Yello Means These Items Can be Optional (Depending on the project)	Links are provided to expand understanding of the item or provide examples of what the item looks like.	Remember This is Highly Based on your Individual Project. Talk to Your Mentor To Verify Your Unique Requirements	NOTE: These Price Points May Vary By Region, Company, and time at which you look to purchase your item. These are only rough guidelines, and you should consult with your mentor for exact pricing. Also note that these are ideal price points you can shoot for, which may represent old or used equipment. There may be times where it would be wise to buy newer equipment at a higher cost to ensure the materials work. All prices are in CAD dollars.	
Item Name	**Description**	**Link**	**Required? And Why?**	**Price For This Item New?**	**How Much Should I Pay?**
Autoclave	Used to sterilize equipment and solutions which will be used to grow your bacteria.	http://www.ebay.com/bhp/mini-autoclave	Yes - often can use a pressure cooker to work as an autoclave. Another way to acquire is one by asking a dentist or other medical professional for a used piece of machinery	$1,000 - $5,000	Free - $750
Centrifuge - large	If you will be spinning down (pelleting) bacteria of 15 mL volume or more (for use with falcon tubes), you may want to invest in a larger centrifuge that is ammenable to these volumes. Centrifuges of this size need to spin to 4000 RPM (rotations per minute) with a RCF (relative centrifugal force) of ~3,000 in order to pellet the bacteria. Please note you do not have to use this.	https://ca.vwr.com/store/catalog/product.jsp?catalog_number=51000-030 and http://www.jove.com/science-education/5019/an-introduction-to-the-centrifuge	No - This is an optional item, and because of the force required to spin these tubes, I would not suggest DIY solutions to this item	$2,000 - $10,000	~$1000
Centrifuge - Mini	Used to pellet (force to the bottom of the tube) bacteria or separate mixtures using centrifugal force.	https://uk.vwr.com/app/catalog/Product?article_number=521-1647 and http://www.jove.com/science-education/5019/an-introduction-to-the-centrifuge	Yes - definitely will require one of these at a minimum to be able to spin down your bacteria and pellet them at the bottom of the tube. This will be used with many kits and other applications	$500 - $5,000	~$200
Computer	Used to access important programs that will be used in molecular biology.		Yes - will need these to access information online	$500-$2,000	~$200
Dish Washer	While not completely necessary, something to clean your equipment in may prove useful.		No - You do not need a dish washer, but you need an area to be able to clean glassware. You should have access to soap and other cleaning supplies	$200 - $2000	~$100
Dry Block Heater	A block which can be used to increase the temperature of tubes from 20 -100 degrees Celsius. This is useful for heating tubes to 37 degrees (Restriction Digest), 42 degrees (transformation), or to high temperatures (~60 degrees - heat inactivation). It is not	http://www.labdepotinc.com/c-569-dry-block-heater.php	No - You can use a waterbath instead of this item for changing temperature. This is simply for convenience sake.	$200 - $600	~$100
Freezer	Must be able to hold a temperature of -20 degrees Celsius to keep solutions cold. It is very important that the freezer does not "cycle". What this means is that the temperature of the freezer must remain constant, such that enzymes or DNA that is stored at this temperature is not constantly being heated and cooled. Regular freezers cycle to prevent frost build up inside of the freezer. Some chest freezers do not have this option, be sure to look into whether yours has this issue.		Yes - Definitely require this item	$100 - $1000	~$100
Fridge	Must be able to hold a constant temperature of 4 degrees Celcius. Use a thermometer to check that your fridge is working accurately and at the correct temperature.		Yes - Definitely require this item	$500 - $3,000	~$100
Fumehood	Safety is incredibly important. See the link for how a fumehood works. A fumehood is used whenever you will be dealing with chemicals that can be easily aerocalized, meaning that they will enter the air quickly. These can be toxic to you, and you should	http://en.wikipedia.org/wiki/Fume_hood and http://www.globalspec.com/learnmore/labware_scientific_instruments/laboratory_safety_equipment/laboratory_fume_hoods	Yes - If you do not have access to one of these in your space, talk to one of your mentors about safety of chemicals.	Variable	Variable
Gas line (bunsen burner)	Gas lines will be required to sterilize some equipment and for aseptic technique when dealing with bacteria. If you do not have access to a gas line that is built into your school you can buy gas tanks to hook up to a bunsen burner to create a flame. It is VERY IMPORTANT that you use the appropriate gas such that you are not producing hazardous fumes (such as carbon monoxide). Always talk to a mentor if you have to use your own gas that is not built into your facility		Yes - If you do not have access to gas lines you can use gas canisters that can be ordered. Please talk to a mentor if you have to go this direction	Variable	Variable
Gel Box and Gel Cast System	Used to run DNA gel electrophoresis	www.bio-rad.com/en-ca/category/nucleic-acid-electrophoresis-blotting and http://www.dnalc.org/resources/animations/gelelectrophoresis.html	Yes - needed to be able to visualize DNA and run gel electrophoresis	$400 - $1,200	~$250
Gel Power supply	Important for gel electrophoresis, it is used to create the voltage difference. It will need to be able to reach and maintain voltages from about 40 V - 120 V and should have an amperage range of at least 100 mA.	http://www.bio-rad.com/en-us/product/power-supplies	Yes - this is a necessary component of the gel electrophoresis system. The purpose of this is to create a constant voltage for your DNA to run into the gel (typically around 80 V). This will likely be an item that you will have to buy. You can DIY this but I would not recommend it due to the voltage you will be dealing with	$600 - $3,000	~$200
Gel Imager	A light generator (usually blue or UV light) that will allow you to visualize DNA from gel electrophoresis. Gel imager units sold by biotech companies are very expensive, and while this type of UV light will provide the best results for your gel, you can use other types of methods to see the DNA. Other methods including using a blacklight or using a blue pen. Look in the DIY section for how to generate some of these.	http://www.lifetechnologies.com/us/en/home/life-science/dna-rna-purification-analysis/nucleic-acid-gel-electrophoresis/e-gel-electrophoresis-system/e-gel-i-mager-system.html and http://genefoo.com/blogs/news/7210826-gelfoo-project	Yes - while the gel imager is not required, you do need a way to visualize your gels. The DNA is stained with a molecule that is fluorescent, and is excited typically within the UV range. You can use a blacklight or another DIY solution (detailed in the link)	$500 - $5,000	~$50
Incubator	Used to heat bacterial plates and other solutions. Usually should be set to 37 degrees Celcius, the temperature that E. coli bacteria grow at. Note that this item can be combined with the shaker item below. You will need a stationary area to grow bacteria on agar plates, and an area of the incubator that rotates to grow bacteria in liquid culture. See the links for examples of incubators and the DIY section for how to put together your own DIY version of an incubator.	http://hackteria.org/wiki/index.php/DIY_Incubator and http://hackaday.com/2013/12/30/diy-incubator-cooks-bacteria-or-yogurt/	Yes - you will require something to grow your bacteria in. This is a very easy DIY project.	$300 - $2,000	~$150
Incubator/Shaker	Used to mix bacterial cultures while growing. Composed of a temperature controlled space with a rotating platform. See above for more information.		Yes - you will require something that rotates to keep your bacteria in motion while they are growing. E. coli are aerobic which means that they need oxygen to grow. If you do not rotate them, they will fall to the bottom of the tube and use up all of the oxygen.	$1,000 - $5,000	~$500
Ice maker (supply of ice)	You will require a way to keep some tubes at around 4 degrees. This is most easily accomplished using crushed ice. While an ice maker machine itself is not required, you may want to invest in some ice cube trays or purchasing ice so that it is available to keep your tubes cold	http://www.kmart.com/search=portable%20crushed%20ice%20cube%20maker?storeId=10151&catalogId=10104&viewItems=50&levels=Appliances&vDropDown=defaultOpt&sLevel=0&redirectType=SKIP_LEVEL	No - This piece is optional and you could use ice cube trays or bagged ice as an alternative.	$100 - $1,000	~$50
Magnetic stir plate/heater	A stir plate is used with a magnetic stirring rod, a plastic piece with a magnetic core that you drop into solutions. This is used to make up solutions that you will require, such as TAE for running gels. The heater feature on this unit is optional, but can be handy if you ever need to heat a solution that you are making.	http://www.ebay.com/bhp/magnetic-stir-plate and http://www.homebrewtalk.com/f51/my-stir-plate-cheap-easy-build-86252/	Yes - this item is one that you can buy from a biotech company relatively cheaply. You can also look into DIYing a solution for it which is listed in the links	$350 - $1,000	~$100
Microwave	Used to dissolve the agarose used in gel electrophoresis and to warm/semi-sterilize some solutions. Remember to never use a lab microwave for food or human consumption!		Yes - this is a relatively cheap item that you can probably find used from a friend or family member.	$100 - $500	~$50
PCR Machine	Used to selectively amplify a sequence of DNA. This item can be costly and is basically a heating/cooling block that tubes can sit into. It ramps up and down in temperature very quickly (about 1 degree per minute) and can go from 4 degrees to 99 degrees	http://loci.wisc.edu/equipment/polymerase-chain-reaction-pcr-machine and http://u20science.org/meganmantel/2013/01/11/what-is-a-pcr-machine-how-does-a-pcr-machine-work/	No - This item is not required in all projects. It is only needed if you plan to use PCR in your experimental plan. If you are unsure if you will need to use PCR for your application, contact your mentor	$1,000 - $5,00	~$600
pH Meter	Used to pH solutions to the correct level. As an alternative you can buy pH strip paper for pHing your solution. It is very important that all of the solutions that you make in the lab are at the appropriate pH for the bacteria to be able to survive.	https://ca.vwr.com/store/catalog/product.jsp?product_id=8932759	Yes - Either a pH Machine or pH strips will be required for your project.	$500 - $2,000	~$10 (strips)
Pipettes	There are many types of pipettes that you can use in the lab. The ones that we are specifically talking about here are micropipettes - devices that move small volumes (1 mL or less) and will be the most important asset in your lab. It is important	http://www.google.ca/imgres?imgurl=&imgrefurl=http%3A%2F%2Fupcycleus.blogspot.com%2F2011%2F02%2Fclock-made-with-bottle-and-pendulum-for.html&h=0&w=0&sz=1&tbnid=IZA1Nu9HKSySM&tbnh=225&tbnw=225&zoom=1&docid=wyAdGJUXeY0zoM&ei=ZJ-	Yes - You will require pipettes. It is important to find good quality pipettes and this may be an item that you will want to spend additional dollars on. A good deal on a pipette is usually around $100 CAD but their price can vary dramatically	$200 - $500 each	~$75 each
Scale	Used to measure reagents for making solutions. There are rough and analytical scales that you can purchase, but it is important that it will read to at least one decimal place per gram (~0.1 g). This should be the minimum size you will need to weigh out for	http://www.globalindustrial.ca/p/packaging/scales/lab-balance/Iseries-digital-lab-scale?infoParam.campaignId=T9A&gclid=CJv_pIGfk7sCFcxAMgod35gA5A&gclsrc=aw.ds	Yes - you will require a balance/scale in order to weight out your reagents and materials	$200 - $2,000	~$100
Spectrophotometer/Fluorometer	This device is used to measure a variety of different things. It uses light that shines into a small container called a cuvette that contains a solution to determine different properties of the solution including turbidity, absorbance, fluorescence, and other	http://publiclab.org/wiki/spectrometer and http://www.instructables.com/id/A-simple-DIY-spectrophotometer/ and http://chemwiki.ucdavis.edu/Physical_Chemistry/Kinetics/Reaction_Rates/Experimental_Determination_of_Kinetcs/Spectrophotometry	No - This is an optional item. If you want to characterize your parts you may need this. It can be very expensive to buy new and therefore you may want to look into DIYing this item. There are some great payable options for buying the equipment	$5,000 - $20,000	~$1000
Vortex Mixer	An agitator for tubes and solutions used to shake tubes at a very fast speed. This is mainly for mixing purposes. It is possible to build one of these machines yourself however it is not completely required. You can mix solutions by pipetting up and down	https://uk.vwr.com/app/catalog/Product?article_number=444-2791	No - this is an optional item.	$100 - $500	~$75
Water Bath	Used to increase or decrease the temperature of solutions for long periods of time. Highly recommend having one of these. There are instructions to DIY your own water bath within the guidebook if you are interested in pursuing this.	http://www.thermoscientific.com/en/search-results.html?keyword=water+bath&countryCode=US&matchDim=Y	Yes - this is a required item. You can DIY this item	$250 - $5,000	~$100
Water filtration system	You will need some clean water in order to make most of your solutions. The water should be distilled for making solutions, and double distilled when working with DNA. More information is available in the guidebook. Make sure you are working with the correct quality of water for each experiment that you want to run. dH2O (single distilled water) is good for any media or large bulk solution that you will use with your bacteria. ddH2O (double distilled water) is needed for any application with pure DNA or enzymes.	http://www.ebay.com/bhp/water-distillation-system	Yes - Because a water filtration system can be quite expensive, it is important to talk to your mentors about your unique situation when it comes to purified water. You can purchase distilled water from biotech companies at a moderate rate, however, it is best if you can produce it yourself. Because you will be using only a small volume of double distilled water for your DNA experiments, it is simple and effective to purchase this from a company.	$1,000 - $10,000	~$500 (bottled pure water)

Equipment - Chemicals and Reagents for Molecular Biology

Name	Description	Size	Catalogue #	Company
10X Sample Loading Buffer	Used for loading DNA electrphoresis gels	5 x 250 uL	CA101414-302	VWR
80-Well PCR Holder and MCT Tube Rack	Used for holding 1.5 mL tubes	Pack of 5	89230-670	VWR
Acetic Acid, Glacial	Reagent for protocols	500ML	CA97064-482	Amresco
Acrodisc® Syringe Filters with Supor® Membrane, Pall® Life Sciences 0.22 um	Used to sterilize materials	Box of 50	CA28143-310	PALL
Agar, Bacteriological	Used to plates to grow bacteria	1KG	CA97064-332	Amresco
Agarose RA	Used to make agarose gels for electrophoresis	100G	CA97064-250	Amresco
Ampicillin Sodium Salt	Antibiotic that can be used to select for your transformed bacteria	5G	CA97061-442	Amresco
Bacterial Spreader-Large	See Small Equipment	1	53800-008	VWR
Bacterial Spreader-Small	See Small Equipment	1	53800-010	VWR
Bench coat	Paper used to cover benches (not required) more for preventing spills	1 roll	51138-500	VWR
BioBrick Cloning Kit	Contains PstI, EcoRI, SpeI, and XbaI (the restriction enzymes), Your T4 Ligase as well. These are all of the enzymes you need to do cloning (put your construct together).	1 Kit	E0546S	NEB
Biorad Pen	This can be used to visualize gels. It does not work well. A blacklight may be more effective	1	#166-0530EDU	Bio-Rad
Calcium Chloride	Used to make cells compotent for transformation	500G	CA97062-590	Amresco
Chloramphenicol USP	Antibiotic that can be used to select for your transformed bacteria (Dissolve it in 100% ethanol!)	50G	CA97061-244	Amresco
D-(+)-Glucose	May be required to make the media your cells will grow in	1KG	CA97061-164	Amresco
ddH2O	Used for DNA or enzyme work. Super pure water.	500 mL	RC91501	Ricca
DNA Ladder 1KB	Used for DNA electrophoresis to determine the size of your DNA bands	500uL	CA97063-490	Amresco
dNTPs	Used for PCR, only required if you are planning to do PCR.	1 box	N0447S	NEB
E.Z.N.A. MiniPrep Kit	Required to extract plasmid DNA from your bacteria.	200 Preps	CA101319-320	VWR
EDTA	Used in gel electrophoresis. EDTA is a chelator and will soak up metal ions in solution. This is used in gel electrophoresis as DNA is normally attached to magnesium (Mg) which needs to be stripped away for the gel to run correctly	500G	CA97061-022	Amresco
Glycerol - Proteomics Grade	Very heavy viscous solution used to make glycerol stocks for long term storage of bacteria	250ML	CA97063-892	Amresco
Glycine - Proteomics Grade	Used as a buffering agent in some solutions (to keep pH stable)	250G	CA97063-736	Amresco
Hydrochloric Acid 6N	This is used to pH solutions	1L	CA97064-760	Amresco
IPTG	If YG is only required if you will be using the lacI promoter to control your circuits. IPTG induces this	1G	CA97061-780	Amresco
KANAMYCIN SULFATE10GM	Antibiotic that can be used to select for your transformed bacteria	10G	CA97061-600	Amresco
Kimwipes	See Small Equipment	Box of 1	470173-504	VWR
Lab Tape	See Small Equipment (Remember you can also use masking tape, it can be autoclaved)	Rainbow Pack	89097-990	VWR
Magnesium Chloride Hexahydrate	Used for chemical compotent cells that you need to make for transformation.	500G	CA97061-356	Amresco
MAXYMum Recovery™ Research-Grade Pipet Tips, Axygen Scientific 1000UL	Used with micropipettes. Make sure to autoclave before use.	Case of 5000	22234-086	AXYGEN
MAXYMum Recovery™ Research-Grade Pipet Tips, Axygen Scientific 10UL	Used with micropipettes. Make sure to autoclave before use.	Case of 20,000	22234-092	AXYGEN
MAXYMum Recovery™ Research-Grade Pipet Tips, Axygen Scientific 200UL	Used with micropipettes. Make sure to autoclave before use.	Case of 10,000	22234-070	AXYGEN
Parafilm	See Small Equipment	1 box	52859-079	VWR
Phusion High Fidelity PCR Kit	This is only required if you will be using PCR in your project with a PCR machine.	50 Reactions	E0553S	NEB
Pipette Bulb	See Small Equipment	1	53497-055	VWR
Razor Blades	See Small Equipment	Pack of 100	55411-050	VWR
Serological Pipettes 10 mL	See Small Equipment	Case of 200	89130-898	VWR
Sodium Acetate Anhydrous, ACS Grade		500G	CA97061-998	Amresco
Sodium Chloride, Molecular Biology Grade	Salt, important for numerous different applciations in your project	1KG	CABDH4534-1KGP	BDH
Sodium Dodecyl Sulfate (SDS)	This is used to lyse cells and to denature proteins.	100 mL	CA97062-964	Amresco
SYBR®Safe	The dye that will stick to DNA that causes it to fluoresce using your gel imager or black light.	400 uL	S33102	Life Technologies
Syringes	Used for filtering see Small Equipment	1 pack	66064-768	VWR
TAQ DNA Polyermase	Only buy if you will be using PCR in your project.	400 Units	M0267S	NEB
Transfer Pipet	Pasteur pipettes see Small Equipment	Pack of 500	414004-001	VWR
Tris-Base	Used as a buffering agent in some solutions (to keep pH stable)	1KG	CA97061-794	Amresco
Tryptone	Used to make LB media	1KG	CA97063-388	Amresco
VWR Black Stripe Autoclave Tape	See Small Equipment	Case of 60	89097-924	VWR
VWR Disposable Microcentrifuge Tubes	You wil use these to contain your bacteria or small volumes of solutions	Case of 5000	89000-028	VWR
VWR® High-Performance Centrifuge Tubes with Flat or Plug Caps, Polypropylene, Sterile 15mL Plug Cap	You can grow your bacterial cultures using these. See Small Equipment. Important to get these pre-sterilized	Case of 500	89039-670	VWR
VWR® High-Performance Centrifuge Tubes with Flat or Plug Caps, Polypropylene, Sterile 50 mL Plug Cap	You can grow your bacterial cultures using these. See Small Equipment. Important to get these pre-sterilized	Case of 500	89039-662	VWR
VWR® Microcentrifuge Tubes, Polypropylene 0.65ML	These are not needed if you do not have a PCR machine that specifically needs this type of tube.	Case of 10,000	87003-290	VWR
VWR® PCR Tubes and Caps 0.2 mL	Used for PCR applications only	1,000 pack	20170-012	VWR
VWR® Petri Dishes, Sterile E-Beam Sterilized	Important that the Petri Dishes come sterilized	Case of 375	89107-632	VWR
Weight Boats	These are expensive, you can always use another method of getting these.	1 case	89106-766	VWR
Yeast Extract, Bacteriological	Used in LB Media	1KG	CA97064-372	Amresco
PCR Purification Kit	Needed if PCR will be used in your project.	1 kit	CA101318-906	VWR
Gel Extraction kit	Used with the gel extraction procedure to isolate DNA from an agarose gel	1 kit	CA101319-300	VWR

NOTE: The Catalogue information here is an example for a potential distributor (mainly VWR). Pricing varies depending on agreements, and you by no means have to use this supplier. Any questions, contact your mentor.

Appendix 3.

MINIMAL HEADER CSS

Patrick Wu

Copy and paste this to your global CSS file and it should remove the photo banner while retaining the control bar (aka '`div#top-section`').

```
/***

Minimal header: removes the search bar and header image and readjusts
font colours in the menus.


Header compiled by Patrick Wu, iGEM Calgary 2011-2013.


Thanks a lot to the 2011 Brown-Stanford and 2012 Lethbridge iGEM teams
for snippets of their code!

Check out their wikis at:

http://2011.igem.org/Team:Brown-Stanford

http://2012.igem.org/Team:Lethbridge

***/


/*hides the search bar and photos*/

#content h1.firstHeading, #contentSub{

      display: none;

}

#p-logo {

      display: none;

}

#searchform {

    display: none;
```

```
}

.left-menu {
      background-color: #555;

}
.left-menu a {
    color: #000;
}

div#top-section{        /*the div containing the entire top bar*/
      background: #DEDEDE;
      border: none;
      height: 1.5em;
      margin-bottom: 0px !important;
      position: fixed;
      width: 100%;
      z-index: 3;
}

#content{
      border: 0;
      margin: 0;
      padding: 0;
      position: static;
      width: 100%;
      z-index: 1;
}
```

```
#search-controls {
	overflow:hidden;
	display:none;
	background: none;
	position: absolute;
	top: 170px;
	right: 40px;
}


div#header {
	width: 975px;
	text-align: left;
	margin-left: auto;
	margin-right: auto;
	margin-bottom: 0px !important;
}

#menubar {
	position: absolute;
	background: none;
	color: black;
	font-family: Roboto, Sans-Serif;
}

.left-menu, .right-menu{
	position: absolute;
	background: none;
	color: black;
}
```

```
.left-menu li a, .right-menu li a {
      color: #000 !important;
      font-size: 0.8rem;
      padding: 0 0.6em 0 0;
}


.left-menu ul li, .right-menu ul li a{
      background: none;
      color: #000 !important;
      font-size: 0.8rem;
}


.left-menu li a:hover, .right-menu li a:hover, .right-menu li
a:visited, .right-menu li a:active {
    color: #000 !important;
      font-size: 0.8rem;
}


div.right-menu{
      z-index: 0;
}


#catlinks{
      display:none;
}


#footer-box{
      background: #DEDEDE;
```

```
	width: 100%;
	margin: 0;
	padding: 0;
	border: none;
}

#f-list li a{
	color: #555555;
	font-family: Roboto, Sans-Serif;
	font-size: 0.8rem;
	text-decoration: none;
}

	#f-list li a:hover{
		text-decoration: none;
	}

/*important for background colours*/
.mediawiki{
	background: #ffffff;
}

/***End minimal header***/
```

GLOSSARY

Active transport - transport of a substance (as a protein or drug) across a cell membrane against the concentration gradient; requires an expenditure of energy (WordNet)

Aliquot - to divide into equal portions.

Amino acid - any of various compounds containing an amino group (NH_2), a carboxylic acid group (COOH), and a distinctive side chain, especially any of the 20 amino acids that link together to form proteins. (The American Heritage Dictionary of the English Language)

Amino acid sequence - the unique sequence of amino acids that characterizes a given protein (Collins)

Anneal - The pairing of complementary DNA or RNA sequences, via hydrogen bonding, to form a double-stranded polynucleotide. Most often used to describe the binding of a short primer or probe (Biology dictionary)

Anti-codon - a sequence of three adjacent nucleotides in transfer RNA that binds to a corresponding codon in messenger RNA and designates a specific amino acid during protein synthesis. (The American Heritage Dictionary of the English Language)

Antibiotic resistance - a form of drug resistance whereby some sub-populations of a microorganism, usually a bacterial species, are able to survive after exposure to one or more antibiotics (Wikipedia)

Aseptic Technique - the technique used to ensure that bacteria or other organisms are transferred in a sterile manner from one container to another. To ensure that no other foreign bacteria or other organisms contaminate your sample.

Bacterial chromosome - the nuclear region of a bacterium, which contains the chromosome but is not limited by a nuclear membrane. (Dorland's Medical Dictionary for health consumers)

Base pair - one of the pairs of chemical bases joined by hydrogen bonds that connect the complementary strands of a DNA molecule or of an RNA molecule that has two strands; the base pairs are adenine with thymine and guanine with cytosine in DNA and adenine with uracil and guanine with cytosine in RNA (WordNet)

Biobrick - DNA sequence which conforms to a restriction-enzyme assembly standard (Wikipedia)

Bioethics - the study of the ethical and moral implications of new biological discoveries and biomedical advances, as in the fields of genetic engineering and drug research. (The American Heritage Dictionary of the English Language)

Biosafety - the maintenance of safe conditions in biological research to prevent harm to workers, organisms, or the environment. Biosafety protects people from bacteria, and vice versa. (Keeping people away from "bad organisms")

Biosecurity - the set of measures taken to limit or counter the threat posed by sudden widespread disease or biological contamination, as from biological warfare, acts of bioterrorism, or pandemic outbreaks, including measures for increasing public safety and preparedness as well as procedures aimed at restricting access to biohazardous materials or to information relating to their production. (The American Heritage Dictionary of the English Language)

Blunt end - an end of DNA in which both strands have the same length

Buffer - a substance capable in solution of neutralizing both acids and bases and thereby maintaining the original acidity or basicity of the solution (Merriam-Webster)

Carbohydrate - an essential structural component of living cells and source of energy for animals; includes simple sugars with small molecules as well as macromolecular substances (WordNet)

Cell - the smallest part of a living structure that can operate as an independent unit (Macmillan)

Cell membrane - a thin membrane around the cytoplasm of a cell; controls passage of substances in and out of the cell (WordNet)

Centrifuge - an apparatus that uses centrifugal force to separate particles from a suspension

(WordNet)

Chromosome - a structure that looks like a very small piece of string and that exists,usually as one of a pair, in the central part of all living cells. Chromosomes contain genes. (Macmillan)

Coding region - part of a gene's DNA that codes for protein

Codon - a specific sequence of three adjacent bases on a strand of DNA or RNA that provides genetic code information for a particular amino acid (WordNet)

Complementary base - either of the nucleotide bases linked by a hydrogen bond on opposite strands of DNA or double-stranded RNA: guanine is the complementary base of cytosine, and adenine is the complementary base of thymine in DNA and of uracil in RNA. (Dictionary.com)

Concentration gradient - a difference in the concentration of a substance on two sides of a permeable barrier (Mosby's Medical Dictionary)

Constitutive promoter - an unregulated promoter from which a gene is transcribed continuously

Controllable promoter - a promoter from which transcription of a gene is regulated by various factors

Daughter strand - the newly made strand resulting from copying the parent strand in DNA replication

Denature - To induce structural alterations that disrupt the biological activity of a molecule. Often refers to breaking hydrogen bonds between base pairs in double-stranded nucleic acid molecules to produce in single-stranded polynucleotides or altering the secondary and tertiary structure of a protein, destroying its activity (online medical dictionary)

Diffusion - the movement of atoms or molecules from an area of higher concentration to an area of lower concentration. Atoms and small molecules can move across a cell membrane by diffusion. (The American Heritage Science Dictionary)

DNA - Deoxyribonucleic acid, a self-replicating material present in nearly all living organisms as the main constituent of chromosomes. It is the carrier of genetic information. (Oxford Dictionaries)

DNA ligase - an enzyme which creates a phosphate bond between 5' and 3' ends of a DNA chain, resulting in a longer chain of DNA. (Free Dictionary)

DNA polymerase - any of various enzymes that function in the replication and repair of DNA by catalyzing the linking of dATP, dCTP, dGTP, and dTTP in a specific order, using single-stranded DNA as a template. (The American Heritage Dictionary of the English Language)

Double helix - The coiled structure of double-stranded DNA in which strands linked by hydrogen bonds form a spiral configuration, with the two strands oriented in opposite directions. (The American Heritage Dictionary of the English Language)

Electrophoresis - The technique of separating charged molecules in a matrix to which is applied an electrical field (Biology dictionary)

Emerging technology - a field of technology that broaches new territory in some significant way, with new technological developments. (Wikipedia)

Enzyme - a protein that catalyzes chemical reactions of other substances without itself being destroyed or altered upon completion of the reactions (Dorland's Medical Dictionary for Health Consumers)

Ethical dilemma - a complex situation that often involves an apparent mental conflict between moral imperatives, in which to obey one would result in transgressing another. (Wikipedia)

Ethics - a branch of philosophy dealing with values pertaining to human conduct, considering the rightness and wrongness of actions and the goodness or badness of the motives and ends of such actions. (Miller-Keane Encyclopedia)

Eugenics - the study or practice of attempting to improve the human gene pool by encouraging the reproduction of people considered to have desirable traits and discouraging or preventing the reproduction of people considered to have undesirable traits. (The American Heritage Dictionary of the English Language)

Eukaryotic cell - a cell with a true nucleus; a cell with a nuclear membrane and organelles (Dictionary.com)

Gene expression - conversion of the information encoded in a gene first into messenger RNA and

then to a protein (WordNet)

Genetic circuit - functional cluster of genes that impact each other's expression (Wikipedia)

genetic code

Genome/genomic DNA - the genetic material of an organism (Merriam-Webster)

Heredity - the genetic transmission of characteristics from parent to offspring. (The American Heritage Dictionary of the English Language)

Human Practices - The social, legal, business, ethical or environmental elements of an iGEM project that identify how the technical components of a project will affect humanity.

iGEM- International Genetically Engineered Machines Competition

Inducible promoter - a promoter activated or undergoing expression only in the presence of a particular molecule or other factor (Merriam-Webster)

Inducing agent - a physical or chemical factor which causes the activation of a

Inoculate - to introduce (as a microorganism) into a suitable situation for growth (Merriam-Webster)

Intellectual freedom - the freedom to hold, receive and disseminate ideas without restriction (Wikipedia)

IPTG - short for isopropylthiogalactoside; compound used to induce protein expression where the gene is under the control of the lac operator (Wikipedia)

LacZ promoter - a well characterized and widely used promoter element from the lactose operon, which is a nucleotide sequence in *Escherichia coli* that controls the synthesis of the enzyme β-galactosidase

Ligation - the joining of two DNA molecules by a phosphodiester bond. (Saunders Comprehensive Veterinary Dictionary)

Macromolecule - any very large complex molecule (WordNet)

Miniprep - a commonly used method to separate and purify plasmid DNA from a bacterial culture

Moral code - a standard of right behavior (Merriam-Webster)

Moral value - concerning or relating to what is right and wrong in human behavior (Merriam-Webster)

mRNA - short for messenger RNA; the form of RNA that mediates the transfer of genetic information from the cell nucleus to ribosomes in the cytoplasm, where it serves as a template for protein synthesis. It is synthesized from a DNA template during the process of transcription. (The American Heritage Dictionary of the English Language)

mRNA transcript - see mRNA

MSDS - Material Safety Data Sheets, a standard that contains important information for how to appropriately deal with a chemical, the safety information on storage and handling, as well as the emergency information in case inappropriate contact is made with the body. Always have MSDS sheets on hand for any chemicals that you are dealing with.

Mutation - any event that changes genetic structure; any alteration in the inherited nucleic acid sequence of the genotype of an organism (WordNet)

Non-sterile - A container, solution, or other component that is contaminated with organisms. These solutions should not be used for any bacterial work since this will contaminate your solutions.

Nucleotide - a phosphoric ester of a nucleoside; the basic structural unit of nucleic acids (DNA or RNA) (WordNet)

Origin of replication - a particular sequence in a genome at which replication is initiated (Wikipedia)

Outreach - guidelines, principles, legislation and activities that affect the living conditions conducive to human welfare. (TheFreeDictionary)

Palindromic sequence - a nucleic acid sequence (DNA or RNA) that is the same whether read 5' (five-prime) to 3' (three prime) on one strand or 5' to 3' on the complementary strand with which it forms a double helix. (Wikipedia)

Parent strand - original DNA strand used as template for the newly made strand in DNA replication

Passive transport - transport of a substance across a cell membrane by diffusion; expenditure of energy is not required (WordNet)

PCR - short for Polymerase Chain Reaction; a technique for amplifying DNA sequences in vitro by separating the DNA into two strands and incubating it with oligonucleotide primers and DNA polymerase. It can amplify a specific sequence of DNA as many as one billion times and is important in biotechnology, forensics, medicine, and genetic research. (The American Heritage Dictionary of the English Language)

Phospholipid - any of various compounds composed of fatty acids and phosphoric acid and a nitrogenous base; an important constituent of membranes (WordNet)

Pipetting - The use of a pipet to draw up liquid from one container and transfer it to another. NEVER use your mouth to pipet, you should use a bulb to transfer solutions.

Plasmid backbone - the sequence beginning with the BioBrick suffix, including the replication origin and antibiotic resistance marker, and ending with the BioBrick prefix. (iGEM)

Plasmid DNA - a small DNA molecule within a cell that is physically separated from a chromosomal DNA and can replicate independently. (Wikipedia)

Plasmid vector - Plasmid most often found in bacteria and used in recombinant DNA research to transfer genes between cells. (The American Heritage Dictionary of the English Language)

Policy and Practices - The new definition of "human practices" defined by iGEM HQ to be more inclusive of all elements of these components of a project.

Polypeptide - a peptide, such as a small protein, containing many molecules of amino acids, typically between 10 and 100. (The American Heritage Dictionary of the English Language)

Prefix- The prefix and suffix contain restriction enzyme cut sites, that can be used to transfer and assemble your parts. (iGEM) The Prefix contains the restriction enzyme cut sites for EcoRI, NotI, and XbaI

Primer - a segment of DNA or RNA that is complementary to a given DNA sequence and that is needed to initiate replication by DNA polymerase. (The American Heritage Dictionary of the English Language)

Prokaryotic cell - a cell lacking a true membrane-bound nucleus (Dictionary.com)

Promoter - a site on a DNA molecule at which RNA polymerase binds and initiates transcription. (Dictionary.com)

Protein - any of a large group of nitrogenous organic compounds that are essential constituents of living cells; consist of polymers of amino acids (Macmillan)

Recombinant DNA technology - the technology of preparing recombinant DNA in vitro by cutting up DNA molecules and splicing together fragments from more than one organism (WordNet)

Reporter gene - a gene that results in an easily detectable product and that is used as a marker to study the activity of another gene with which its DNA has been combined

Repressor - a protein that binds to an operator, blocking transcription of an operon and the enzymes for which the operon codes. (The American Heritage Dictionary of the English Language)

Restriction cut site - The DNA sequence recognized and cleaved by a restriction endonuclease (Glossary of Drug Discovery and Development)

Restriction endonuclease - also called restriction enzyme; any of the enzymes that cut nucleic acid at specific restriction sites and produce restriction fragments; obtained from bacteria (where they cripple viral invaders); used in recombinant DNA technology (WordNet)

Restriction enzyme - also called restriction endonuclease; any of the enzymes that cut nucleic acid at specific restriction sites and produce restriction fragments; obtained from bacteria (where they cripple viral invaders); used in recombinant DNA technology (WordNet)

Restriction enzyme site (cut site) - the specific site at which a restriction enzyme will cleave DNA (WordNet)

Ribosome - a structure composed of RNA and protein, present in large numbers in the cytoplasm of living cells and serving as the site for assembly of polypeptides encoded by messenger RNA. (The American Heritage Dictionary of the English Language)

Ribosome binding site (RBS) - a sequence on mRNA that is bound by the ribosome when initiating protein translation. (Wikipedia)

RNA - a nucleic acid present in all living cells and many viruses, consisting of a long, usually single-stranded chain of alternating phosphate and ribose units, with one of the bases adenine, guanine, cytosine, or uracil bonded to each ribose molecule. RNA molecules are involved in protein synthesis and sometimes in the transmission of genetic information. Also called ribonucleic acid. (The American Heritage Dictionary of the English Language)

RNA polymerase - a polymerase that catalyzes the synthesis of a complementary strand of RNA from a DNA template, or, in some viruses, from an RNA template. (The American Heritage Dictionary of the English Language)

Social policy - guidelines, principles, legislation and activities that affect the living conditions conducive to human welfare. (TheFreeDictionary)

Staggered (sticky) end - an end of DNA in which one strand of the double helix extends a few units beyond the other (WordNet)

Start codon - The codon at which translation of mRNA into protein starts, encoding the first amino acid. (Wiktionary)

Stop codon - a sequence of three RNA nucleotides (A, C, G or U) that instruct the synthesis, or translation, of a protein to stop. (Wiktionary)

Suffix-The prefix and suffix contain restriction enzyme cut sites, that can be used to transfer and assemble your parts. (iGEM) The Suffix contains the restriction enzyme cut sites for SpeI, NotI, and PstI

Supernatant - The soluble liquid &action of a sample after centrifugation or precipitation of insoluble solids (Biology dictionary)

Synthetic biology - The application of genetic engineering to generate modified or even completely new forms of life. Distinguished from older methods of transgenics by the use of either outright synthesised DNA or DNA which has been modified heavily to become more predictable and standardised. A heavy focus on modular design of genetic "parts" makes synthetic biology more of an engineering discipline than a form of pure science (Wiktionary)

T7 promoter - promoter sequence with high affinity for the RNA polymerase from the T7 bacteriophage (a virus that infects bacteria)

T7 RNA polymerase - the RNA polymerase from the T7 bacteriophage

Terminator - a section of nucleic acid sequence that marks the end of a gene or operon in genomic DNA during transcription. (Wikipedia)

Theory of evolution by natural selection - change in the genetic composition of a population during successive generations, as a result of natural selection acting on the genetic variation among individuals, and resulting in the development of new species. (The American Heritage Dictionary of the English Language)

Transcription - the process of constructing a messenger RNA molecule using a DNA molecule as a template with resulting transfer of genetic information to the messenger RNA (Merriam-Webster)

Transformation - genetic modification of a cell by the uptake and incorporation of exogenous DNA (Merriam-Webster)

Translation - the process whereby genetic information coded in messenger RNA directs the formation of a specific protein at a ribosome in the cytoplasm (WordNet)

tRNA - short for transfer RNA; one of a class of RNA molecules that transport amino acids to ribosomes for incorporation into a polypeptide undergoing synthesis. (The American Heritage Dictionary of the English Language)

UTR - either of two sections, one on each side of a coding sequence on a strand of mRNA. If it is found on the 5' side, it is called the 5' UTR (or leader sequence), or if it is found on the 3' side, it is called the 3' UTR (or trailer sequence). (Wikipedia)

WHMIS - Workplace Hazardous Materials Information System, the organization that in Canada regulates all workplace safety. These regulations must be followed within the laboratory space to ensure a safe working environment. If you are not in Canada, be sure to consult your countries workplace safety organization to determine the appropriate regulations you must adhere to.

Wiki - Website designed by the team to present their project and hosted on the igem.org webiste.